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Journal of DENTAL RESEARCH

Journal of DENTAL RESEARCH

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This *Journal* is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The *Journal* publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this *Journal*.

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Editorial Views and News

The large number of new members elected at the last annual I.A.D.R. meeting in March has already and will continue to have an impact upon the Association as well as its divisions and sections. It is already apparent that more local sections will be activated or reactivated in the near future. As soon as there are the required ten active members who can meet regularly in an area, they should elect officers and send notice of such a new section to the Secretary-Treasurer and the Editor. The latter person will be extremely happy to list such sections or any changes in existing sections on the appropriate page of the next issue of the *Journal*.

When thirty or more I.A.D.R. members in any nation or group of nations on the same continent organize and hold meetings, they may constitute a division of the Association with the approval of the Council. While growth in mere numbers is not necessarily growth in quality, it is good to observe that we are *not* a static scientific society.

The A.I.B.S.—As members who attended the business meeting of the I.A.D.R. in March recall (see the Proceedings in this issue), the American Institute of Biological Sciences, of which the I.A.D.R. has been a member for several years, has a service to which our Association is subscribing. This service will operate the routine affairs of the Secretary-Treasurer's office and relieve Dan and Marie Burrill of the countless details with which they have been burdened over the years. Thus at the last meeting the Council appointed an Assistant Secretary-Treasurer, Howard F. Campbell, of the A.I.B.S. in Washington, who will supervise all routine I.A.D.R. affairs other than *Journal* activities.

"For Those Who Read."—Rather frequently Gerald J. Cox, professor of dental research at the University of Pittsburgh, expounds some very stimulating concepts. He has contributed the following essay for the serious consideration of *Journal* readers.

"The method of fractional page citation herein described is applicable to printed, typed, and hand-written pages, and also to tables, plates and even lantern slides of such diffuse nature as histological sections. It has been used for oral instruction to students for cross-referencing textbooks.

"The method of fractional page citation used in ***Chemical Abstracts,*** upon which this method is based, provides efficient guidance for finding items listed in the index or referred to in abstracts or elsewhere. The fractions of pages were listed down the center of the two-column page, beginning with Volume 28, and these numbers were attached to the column numbers in the index as superscripts. Beginning with Volume 41 the letters *a* to *i* were used. For the diverse uses listed above, return is made to numbers, as notes made in reading are generally hand-written.

"Any page can be judged readily in thirds with sufficient accuracy, and if a decimal fraction from 0.1 to 0.9, inclusive, is appended to the page number, the citation can be quickly found. Thus 67.7 indicates location in the first third of the last third of page (or column) 67.

"For a two-column page with pages instead of columns numbered, citations to the right-hand column may be made by appending 'r' to the number, such as 67.7r. No letter need be appended to indicate the left-hand column, and there may be confusion of the number '1' with the letter 'l.' Capital letters may be used for certainty of the column indicated. For three-column pages, the center column can be identified by appending the letter 'c.' These letters, of course, are derived from the English language. Similar usage for other languages is obvious. The decimals may be attached to Roman numerals if used in pagination.

"For pages not completely filled with printed material, citation by the above-described plan is still possible, the understanding being that fractions of the whole page are meant. If there is only a little material on the page, the fractional citation may not be of any advantage."

—F. J. O.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Thirty-ninth Annual Meeting, March 23, 24, 25, and 26, 1961
Hotel Statler, Boston, Massachusetts

Abstracts of papers for presentation at the thirty-ninth annual meeting of the I.A.D.R. in Boston, Massachusetts, March 23, 24, 25, and 26, 1961, must be submitted on *special forms provided by the Association.*

Abstracts must fulfil the requirements stated on the form and must be fully ready, in form and substance, for inclusion in the preprinted abstracts. The preprinted abstracts will be prepared photographically from the abstracts as submitted—no editing or corrections will be possible. Abstracts not in proper form will be returned to the authors if time allows.

The abstracts of papers presented at the meeting will be formally published in the *Journal of Dental Research* after the meeting.

The deadline for receipt of abstracts will be *December 1, 1960*. Mail all abstracts to: James A. English, Capt. DC USN, Chairman of Program Committee, Office of Naval Research, Building T-3, Navy Department, Washington 25, D.C.

DAN Y. BURRILL
Secretary-Treasurer

A Method for the Evaluation of Occlusal Caries in the White Rat

HARRY BLECHMAN, OM P. GUPTA,* and HENRY A. BARTELS

Department of Microbiology and the Murry and Leonie Guggenheim Foundation, Institute for Dental Research, New York University College of Dentistry, New York

A number of methods for scoring caries in the molar teeth of experimental animals have been published.[1-7] Rosebury, Harshan, and Foley[1] devised a system for recording fissure caries based on the examination of decalcified sections. Schamp and Leicester[2] defined twelve stages of fissure caries in rat molar teeth made translucent with phenol. Cox, Dodds, Dixon, and Matuschak[3] reported a detailed system for evaluating and recording occlusal and fissural cavities. The jaws were stripped of soft tissue, dried, and inspected at predetermined depths following buccolingual grinding. Although the method provides for the detection of caries at various levels, it is time-consuming and the scores are quantitatively unrelated to each other in terms of tooth loss. Simpler methods, often less precise, have found favor with some investigators. Shaw, Schweigert, McIntire, Elvehjem, and Phillips[4] devised a simple and readily reproducible system for scoring fissure caries. Sognnaes[5] defined five stages of occlusal caries, which provided a rough measure of the total occlusal area destroyed. Schlack, Howell, and Taylor[6] ground teeth occluso-cervically in a horizontal plane, examined them under a wide-field binocular microscope, and scored them by a simple procedure. Johansen and Keyes[7] published an excellent review of these and other methods for the examination, recording, and scoring of carious lesions in rodents.

In the course of evaluating the extent of dental caries in intact and sialoadenectomized albino rats, it appeared to us that the available scoring methods are not sensitive enough to differentiate quantitatively between occlusal and fissural lesions of varying degrees of intensity. Because of the wide range in variability in the carious process, it is necessary to express findings in more precise terms, in order to make valid comparisons within and between groups of animals. We feel that available methods could be made more critical in their preciseness by further modifications. The purpose of this paper is to describe a method for evaluating and scoring occlusal caries in albino rats which is readily duplicated, does not involve grinding or slicing teeth, and in which the severity scores correlate with the amount of surface area involved.

MATERIALS AND METHODS

Preparation of jaws for scoring.—The animal heads are placed in 95 per cent ethyl alcohol for a minimum of 2 days for fixation. The jaws are then stripped of soft tissues

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and muscle, and the mandibular arch is separated into two quadrants. The maxillary arch and the two mandibular quadrants may be mounted in albastone* for convenient manipulation and examination (Fig. 1).

A No. 1 Kerr or No. 13 Koch root-canal reamer bent at an angle of 60° about 2 mm. from its tip is used as explorer to remove impacted debris from the occlusal sulci. If care is used in manipulation, occlusal enamel fracturing is minimal, and a clean visual field is readily obtained. Staining the molar teeth with a drop of dilute basic fuchsin facilitates examination of carious lesions.

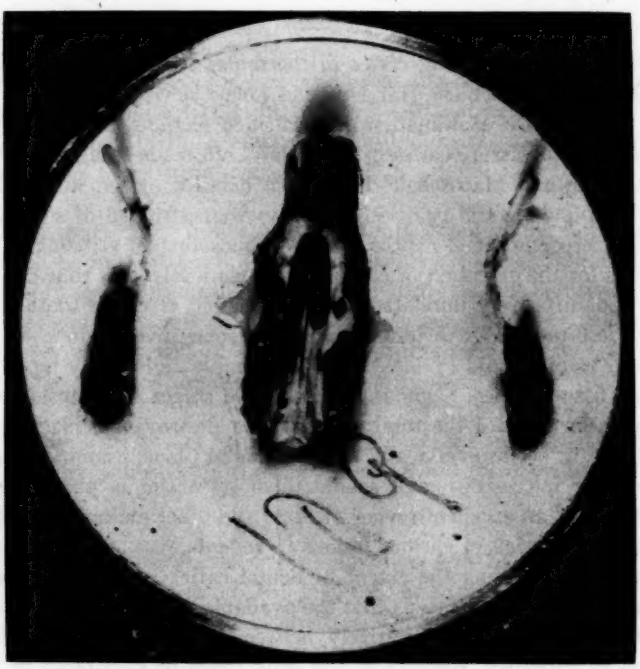

FIG. 1.—The mounted jaws in albastone

The cleaning of the occlusal surfaces and the scoring of carious lesions are performed under a wide-field dissecting microscope at a magnification of 18×.

Method of scoring.—The following method has been evolved on the basis of the careful, repeated examinations of over 2,000 rat heads.

The carious lesions affecting the sulci and the cusps are separately scored in an ascending scale from 1 to 4 as described below.

In the sulcal caries, scores of 1, 2, and 3, depending on the extent of the lesion and the amount of tooth structure involved, are assigned to the sulcal lesions.

Score 1—Pitting: One or more discrete lesions at the base of the sulcus.

Score 2—Spreading: coalescence of two or more discrete opaque lesions with deepening and rounding of the base of the sulcus and carious involvement of the lower half of the approximating enamel walls.

* The S. S. White Dental Manufacturing Company, Philadelphia, Pa.

Score 3—Undermining: extensive widening of the base of the sulcus with undermining of enamel and extension into the dentin.

The carious lesions involving cusps are assigned scores of 1–4, depending on the amount of tooth structure destroyed. A cusp lacking one-fourth of its surface area is scored as 1; half missing is scored or as 2; three-fourths missing as 3; and the loss of an entire cusp receives a score of 4. Where special situations exist the scores are modified in accord with the text that follows.

Scoring of individual molars of each quadrant.—In maxillary molars one quadrant is divided into 15 cuspal areas and 6 sulci (Fig. 2).

The first molar presents two sulci (f_1, f_2) which may receive a score of 1–3, and seven cusps (1–7), which are assigned a score of 1–4, depending on the amount of tooth structure lost. A maximum score of 34 is obtained upon the complete destruction of the tooth.

The second molar presents two sulci (f_3, f_4) which are scored 1, 2, or 3, and five cusps (8–12) which are scored 1–4. A maximum score of 26 is obtained if the tooth is completely destroyed.

The third molar presents two sulci (f_5, f_6) and three cusps (13–15). Because these sulci are relatively small in both depth and length, they are scored either 1 or 2. The cusps are scored from 1 to 4. A maximum score of 16 is obtained when the tooth is completely destroyed.

Since the maxillary arch presents six molars, a total score of 152 is obtained following total carious destruction.

In the mandibular molars one quadrant is divided into 17 cuspal areas and 8 sulci (Fig. 3).

The first molar presents three sulci (f_1, f_2, f_3) and seven cusps (1–7). Since sulci f_1 and f_3 are relatively small, a score of 1 or 2 is assigned. Sulcus f_2 is assigned a score of 1–3. All cusps are scored 1–4, with the exception of cusp 7, which is given the score of 1–2. Total destruction of this molar results in a score of 33.

The second molar presents three sulci (f_4, f_5, f_6) and six cusps (8–13). Sulcus f_4 is assigned a score of 1, f_5 a score of 1–3, and f_6 a score of 1 or 2. Cusps 8–12 are scored from 1 to 4, and cusp 13 is scored 1–2. A maximum score of 28 is obtained.

The third molar presents two sulci (f_7, f_8) and four cusps (14–17). The sulcus f_7 is given the score of only 1, while sulcus f_8 receives a score of 1 or 2. Cusp 14, because of its small area, is assigned a score of 1 or 2. Cusps 15–17 are given scores of 1–4. Complete destruction of this tooth gives a score of 17.

Since the mandibular arch presents six molars, a total score of 156 is obtained following total destruction of all the mandibular molar crowns. Complete destruction of the mandibular and maxillary molars gives a total score of 308. The sum of the scores is a measure of the amount and extent of the carious lesions.

RESULTS

Figure 4 will serve to illustrate the application of the scoring method outlined above.

Sulcus f_1 in Figure 4 is scored as 2, f_2 as 3, and f_3 as 2, giving a total sulcal caries score of 7. In evaluating cuspal caries, cusps *1, 2, 3,* and *4* in relation to sulcus 1 are scored 1 each, giving a total of 4. In relation to f_2, cusps *3, 4, 5,* and *6* are assigned scores of 1 each, giving a total of 4. Cusps *5, 6,* and *7* in relation to f_3 are given the

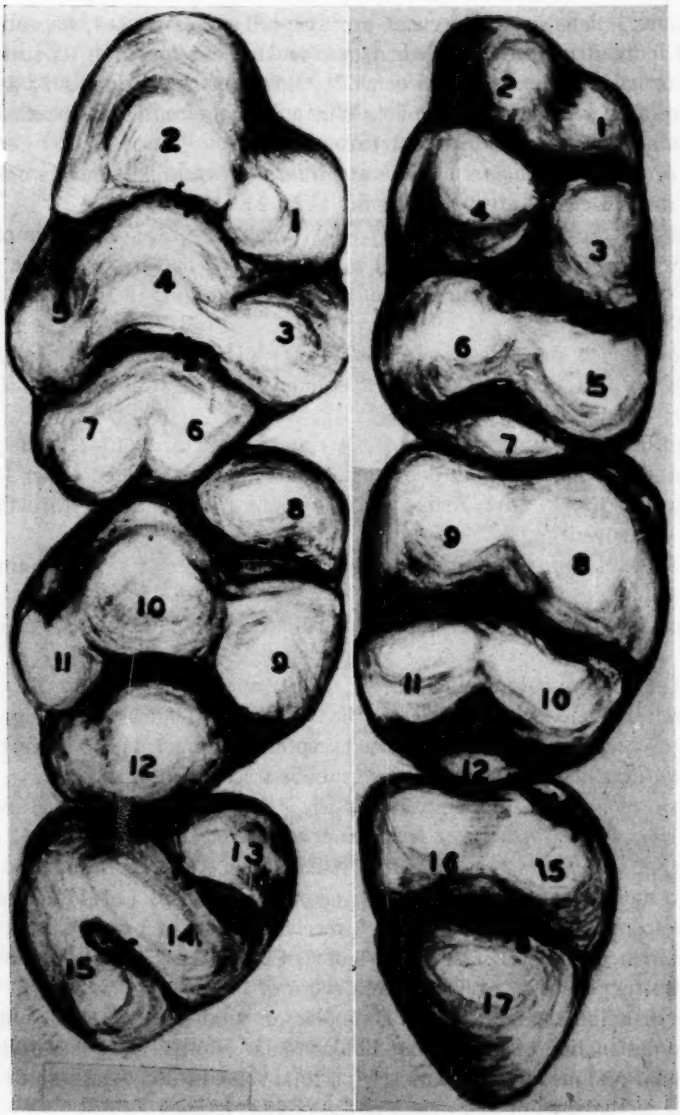

FIG. 2 FIG. 3

FIG. 2.—One quadrant of the maxillary molars divided into 15 cuspal areas and 6 sulci
FIG. 3.—One quadrant of the mandibular molars divided into 17 cuspal areas and 8 sulci

scores of 0, 0, and 1, respectively, giving a total of 1. Thus the total cuspal caries score for the first molar is 9. The sum of the sulcal caries score and cuspal caries score (7 + 9) is 16, which is the total caries score for the first molar.

The second and third molars are evaluated in a similar manner, and a total score is obtained for the quadrant.

The afore-mentioned scoring method was used to score 120 heads by three examiners, and the scores obtained did not differ significantly from each other. Further-

FIG. 4.—Carious mandibular molars

TABLE 1

DIMENSIONS OF MOLARS AND MAXIMUM CARIES SCORES

	Length (Mm.)	Width* (Mm.)	Occlusal Surface Area (Sq. Mm.)	Maximum Caries Score Obtainable
Maxillary:†				
First molar	3.4	2.2	5.27	34
Second molar	2.5	2.2	3.41	26
Third molar	1.75	1.7	2.02	16
Mandibular:‡				
First molar	3.1	1.8	4.21	33
Second molar	2.1	2.1	3.26	28
Third molar	2.1	1.65	2.50	17

* Largest buccolingual dimension.

† The over-all length of the molars in maxillary arch is 6.9.

‡ The over-all length of the molars in mandibular arch is 7.0.

more, the scores were reproducible from one inspection to another by the same examiner. After a careful review of the available occlusal caries scoring methods, it was felt that the above-mentioned procedure consumed less time and effort and at the same time provided greater accuracy.

The average tooth length, average occlusal surface area, and maximum severity score for each of the mandibular and maxillary molars are given in Table 1. A high correlation between surface area and the score for total caries distribution ($r_4 = 0.918$) was obtained, and, on plotting these data, a regular and linear relationship was found.

SUMMARY

A system for recording occlusal caries in the albino rat has been described. The method provides for a high degree of duplicability and sensitivity.

The maxillary molars of one quadrant are divided into 15 cuspal areas and 6 sulci, and the mandibular molars of one quadrant into 17 cuspal areas and 8 sulci. Sulcal caries are scored as 1, 2, or 3, and values of 1–4 are assigned to the cusps. A total severity score is available for each tooth, each quadrant, and each arch.

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Ecological Aspects of Human Parotid Saliva
I. *The Existence of Constant rH Values*

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High oxidation-reduction potentials (+248 to +394 mv) of fresh saliva were obtained by Eisenbrandt;[1] and Richardson[2] claimed that these were too high for the growth of numerous anaerobic of micro-aerophilic bacteria in the mouth. On the other hand, Eggers-Lura[3] reported that lower oxidation-reduction potentials were representative of caries-active groups, whereas higher potentials were those of caries-resistant groups. However, Wessinger[4] indicated that the potential of the saliva does not remain stationary at such a high figure after the sample has been taken. According to Eisenbrandt,[5] the potential migrates irregularly toward the negative side in due time. It is also known that the oxidation-reduction potential is not independent of the pH under consideration, because the electrode potential may be regarded as being established by the equilibrium between hydrogen ions and hydrogn atoms. Eisenbrandt[6] obtained varied pH values from saliva, 5.28–7.42, and found that generally it moves toward the alkaline side when allowed to stand. Schmidt-Nielsen[7] found a constant pH in resting parotid saliva. It is also obvious that reducing or oxidizing the intensity of the system depends not upon the electrode potential but on the molecular H_2 pressure. Therefore, if saliva was discussed with respect to the bacterial respiration in it, direct expression of H_2 pressure should be more convenient.

Wurmser[8] stated in his monograph that various kinds of oxidation-reduction systems often show that constant H_2 pressure is independent of the varying pH of the medium. In this case, the reciprocal of the logarithm of H_2 pressure may be expressed by the term "rH," which was originally introduced by Clark and Cohen.[11] Unfortunately, the greatest advantages of this rH scale have not been appreciated. After a brief period of its use, this method of reporting has been discontinued, and the majority of workers now express their results in the less satisfactory form of electrode potentials. Very recently, however, Dixon[9] showed that if the data are expressed in rH, a series of simple and most convenient rules emerges to which there is no counterpart when they are expressed on any other scale.

EXPERIMENTAL METHODS

In situ measurements of the pH and Eh of parotid saliva were obtained by use of the Hayashi-Hayakawa's saliva collector.[10] This instrument consists of an inner chamber for receiving saliva from the opening of the parotid duct and an outer cham-

This paper was presented at the tenth annual meeting of the Japanese Stomatological Society, Nagoya, 1956.

Received for publication May 1, 1959; revised by authors December 28, 1959.

ber connected to an aspirator which produces negative pressure in the chamber (Fig. 1). The saliva thus collected is drawn into the capillary tube (*K*) which is enclosed by the electrode (*H*) for the measurement of pH. A platinum electrode (H_2), a calomel electrode (*B* and *C*), and a small thermometer (T_1) are located within the incubator. The entire measuring system within the incubator was maintained at a constant temperature of 37° C. The system was connected to a potentiometer which was placed on the outside of the incubator (Fig. 2, *B*). The surface of the platinum electrode was polished with sandpaper and washed thoroughly with distilled water before each measurement.

The mouth was rinsed with sterile tap water, and the saliva collector was fixed at the opening of the parotid duct. Salivation was stimulated by allowing the patient to chew on sour candy. The saliva thus produced was led into the measuring system, and the initial 3 ml. were discarded. Both the source of entry (*I*) and the outlet (*O*)

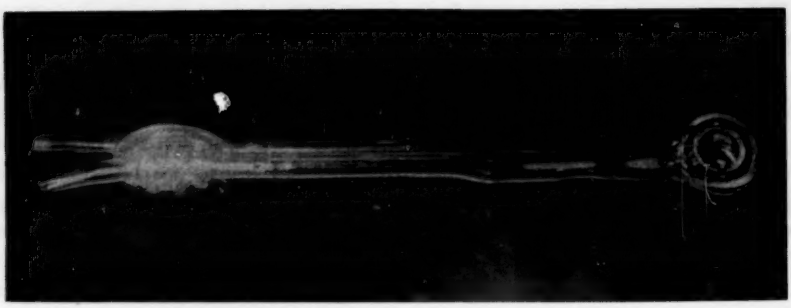


FIG. 1.—Hayashi and Hayakawa's saliva collector

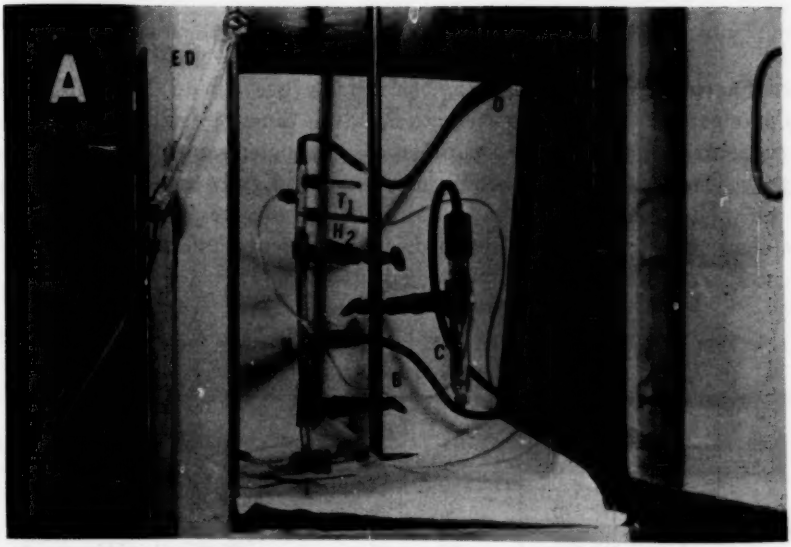
were closed to avoid escape of CO_2 and water vapor from the saliva. Readings on the potentiometer were taken every 10 minutes.

The saliva remaining in the collector was drained into a small sample cup 20 mm. in diameter. The pH and Eh of the saliva were measured by dipping a small bulb-shaped glass electrode, a platinum electrode, and a KCl agar bridge from a calomel electrode into the cup.

RESULTS

1. *Simultaneous measurement of Eh and pH of parotid saliva in situ.*—Saliva taken from 33 adults ranging in age from nineteen to forty-four years was examined. These included both males and females, whose pH's ranged from 7.25 to 7.81 and their Eh's from +317 to +371 mv, as shown in Table 1. Examination of these values indicate that the higher the potential of the saliva, the lower is the pH.

2. *Eh of the saliva when its pH was modified with the addition of acid or alkali.*—After the pH of the parotid saliva was modified by the addition of small amounts of lactic acid or ammonia, the resulting Eh was measured at 37° C. The values obtained are listed in Table 2. These values were combined with those of Table 1 and plotted against pH values, as shown in Figure 3.



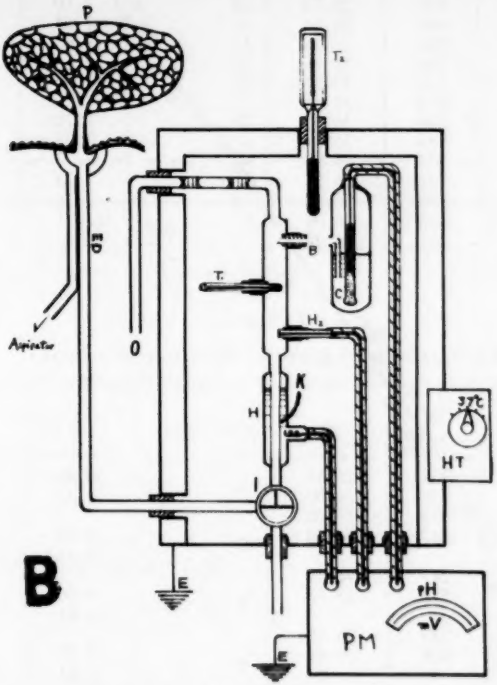


FIG. 2.—Saliva apparatus within incubator (*A*) and its schematic view (*B*). *P* = parotid gland; *ED* = Hayashi-Hayakawa saliva collector; *H* = capillary glass electrode; H_2 = platinum electrode; T_1, T_2 = thermometers; *C* = calomel electrode; *B* = KCl-agar bridge; *PM* = potentiometer; *HT* = thermostat; *E* = earth.

DISCUSSION

According to Schmidt-Nielsen,[7] the considerable rise (up to 2 pH units) in the pH of pure parotid saliva, which averaged 5.81, was caused by either gustatory, olfactory, or mechanical stimulation. The pH of the stimulated saliva in his case coincides with the values shown in Table 1. Eisenbrandt[1] and Eggers-Lura[12] obtained slightly lower Eh values from mixed saliva, +301 and +310 mv, respectively. The variation in the above values from those obtained by the present authors may be due to the differences in specimens and sampling methods.

TABLE 1

PH AND EH VALUES OF PAROTID SALIVA

Specimens	pH	Eh (mv)	rH	Specimens	pH	Eh (mv)	rH
1	7.25	371	26.56	18	7.54	328	25.74
2	7.25	370	26.56	19	7.55	357	26.70
3	7.35	366	26.58	20	7.60	350	26.50
4	7.37	368	26.70	21	7.60	348	26.50
5	7.37	358	26.38	22	7.60	347	26.48
6	7.39	366	26.66	23	7.63	337	26.20
7	7.40	352	26.24	24	7.65	323	25.80
8	7.43	366	26.74	25	7.65	317	25.60
9	7.43	354	26.36	26	7.66	338	26.30
10	7.43	352	26.28	27	7.70	341	26.48
11	7.46	359	26.52	28	7.70	323	25.88
12	7.47	345	26.14	29	7.73	343	26.60
13	7.48	337	25.90	30	7.73	340	26.52
14	7.50	341	26.08	31	7.73	321	25.94
15	7.52	358	26.60	32	7.75	318	25.82
16	7.53	336	26.06	33	7.81	323	26.12
17	7.54	343	26.22				

TABLE 2

EH RESPONSE TO ALTERED PH OF PAROTID SALIVA

Specimens	pH	Eh (mv)	rH	Specimens	pH	Eh (mv)	rH
1	5.02	477	25.54	6	8.89	256	26.10
	3.33	559	24.80		4.37	518	25.54
	3.76	538	25.00				
	9.02	247	26.04	7	8.80	259	26.00
2	9.06	247	26.14		5.17	468	25.54
	4.48	518	25.78	8	9.06	259	26.52
3	9.19	250	26.50		4.05	548	25.90
	4.84	508	26.18	9	9.17	238	26.08
4	9.24	246	26.48		5.83	443	26.06
	5.00	481	25.62	10	8.60	259	25.60
5	8.75	253	25.72		4.44	518	25.68
	6.45	388	25.50				

The relationship obtained in this study was fairly linear, as shown in Figure 3. When calculated by the Eh-pH equation, the straight line,

$$Eh = 768 - 56.6\ pH,$$

was obtained by means of the least-squares method. The correlation coefficient and F_0 were estimated to be 0.991 and 2,968 respectively; these figures indicate highly significant linearity of the Eh-pH curve.

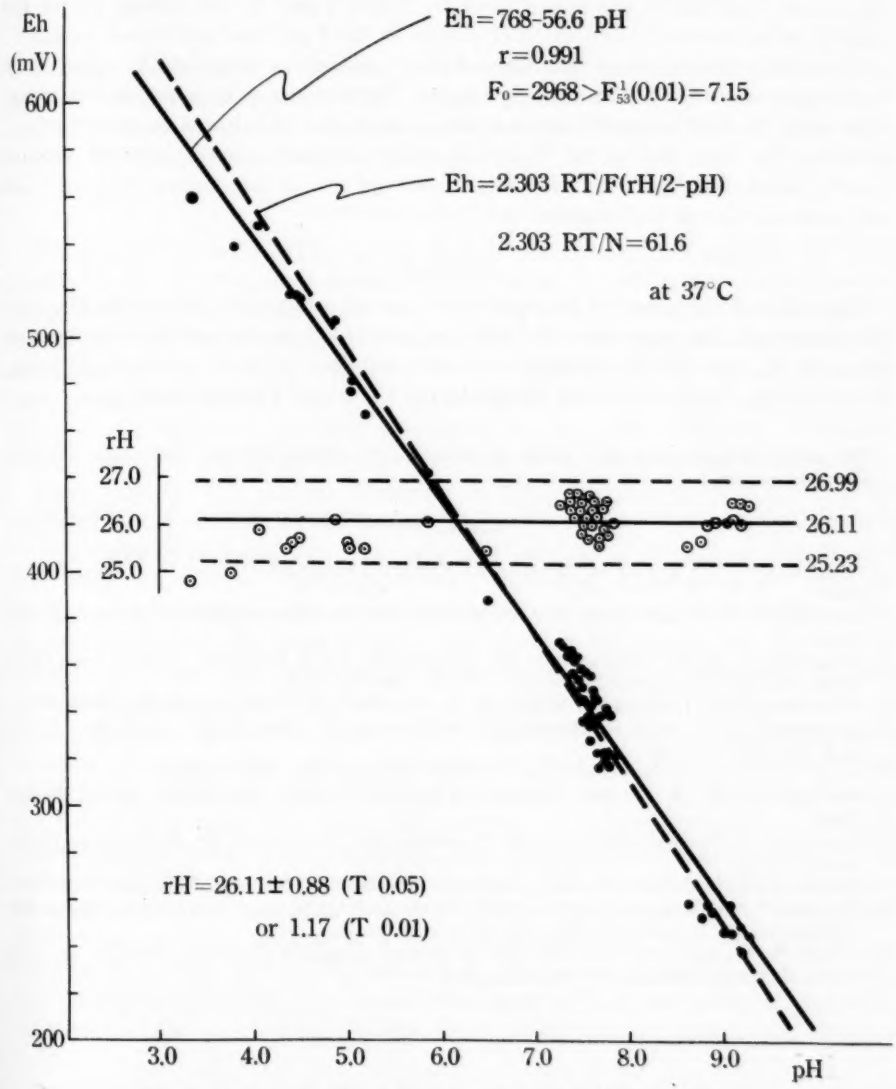


FIG. 3.—Combined values of Tables 1 and 2 plotted against pH values

Clark and Cohen[11] indicated that a reversible oxidation-reduction system, in which one electron only and only one dissociation constant are concerned, is theoretically expressed by the equation

$$\mathrm{Eh} = 2.303\frac{RT}{F}\left(\frac{\mathrm{rH}}{2} - \mathrm{pH}\right).$$

The slopes of both equations may be similar, since $2.303RT/F$ in the latter equation is 61.6 (at 37° C.), which is very close to 56.6 of the equation we obtained. Applying the second equation to the values listed in Tables 1 and 2, the average rH of the parotid saliva was calculated as 26.11 $\pm$0.88 at the 5 per cent confidence level.

This study demonstrated that parotid saliva exhibits a constant rH value of 26 in a wide range of pH's. This strongly suggests the existence of high oxidative activity. The initial rH level of parotid saliva is very close to that of catechol or cytochrome C, involving the final step of the biological oxidation chain. Such a high rH level of parotid saliva, therefore, may play some important role in oral microbial ecology and the keratinization of oral mucosa.

SUMMARY

The pH and Eh values of the stimulated parotid saliva, collected in the Hayashi-Hayakawa collector, were measured with the capillary glass electrode and the platinum electrode. In spite of the variation in results obtained, a fairly constant rH value, 26.11 $\pm$ 0.88, can be calculated by employing Clark and Cohen's equation.

The authors wish to thank Mr. Toshio Kawatomari, 406 Medical General Laboratory, APO 343, for English rewriting.

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Prevalence of Periodontal Disease in a Hospitalized Population

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This study was designed to measure the prevalence of periodontal disease in a hospitalized population and to determine whether persons with confirmed diagnoses of specific diseases demonstrate a greater prevalence of periodontal disease than those who are in relatively good health.

EXPERIMENTAL METHODS

Description of sample.—The sample studied was drawn from patients who were hospitalized at the Veterans Administration Hospital, Brooklyn, New York. Veterans comprised a large proportion of the total male population, but eligibility for hospitalization depended upon whether or not disabilities were incurred during military service or low economic status. Every patient admitted to the hospital received a complete physical examination, chest X-ray, urinalysis, and blood count as a very minimum; most patients received even more extensive examination. The oral examination was completed by the Dental Service as an integral part of the physical examination.

From July 1, 1957, through December 31, 1958, a total of 9,241 patients was admitted to the hospital, of which 7,325 were seen by a staff dentist. Patients who were not seen by a dentist were those who were seriously ill throughout their hospital stay, those admitted for contagious diseases, those hospitalized for a few days, and those discharged unexpectedly or against medical advice. Complete oral examination in the dental clinic, including a full-mouth series of radiograms, was provided for 4,832 patients, and these comprised the sample under study. Of this number, 838 were edentulous, leaving 3,994 who had one or more teeth present in their mouths and were potentially susceptible to periodontal disease. There was no statistical breakdown for sex or race, since more than 90 per cent of all admissions to the hospital and of all patients examined were white males and less than 0.5 per cent were females.

Calibration of examiners.—Analysis of the various types of measurements of periodontal disease[1-16] led to the conclusion that the periodontal disease rate* (PDR) was reasonably precise and practical for the study of large populations. The definitions of what constituted "periodontal disease" and the application of the measurement have been carefully outlined in a previous publication.[17] In order to determine whether the criteria were clearly understood and whether different dentists examining the same patients would reach similar conclusions in their evaluation of periodontal disease, the following test was applied. On a specific day all patients who appeared

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* Proportion of teeth affected by periodontal disease.

at the dental clinic for oral examination were examined independently by all members of the dental staff. The findings (PDR) of the seven dentists on duty agreed very closely when the patient demonstrated extensive or minimal symptoms of periodontal disease, but, when periodontal disease was of moderate severity, agreement was less consistent. Although the differences between the average findings were not statistically significant (critical ratio = <2), examiner training was nevertheless continued in order to reduce differences still further prior to undertaking a large-scale survey.

After a short period of practice and conference among the examiners, another test was scheduled. On a designated day, 26 patients appeared at the dental clinic for examination and were examined independently by the same seven dentists. The individual PDR's still varied from dentist to dentist, particularly among patients with moderate periodontal disease, but the averages all fell within a very narrow range of variation (Table 1). The highest average PDR was obtained by the periodontist in the group and the lowest by one of the staff dentists. However, the difference between

TABLE 1

AVERAGE PERIODONTAL DISEASE RATE IN 26 RANDOMLY SELECTED PATIENTS INDEPENDENTLY DETERMINED BY 7 DENTISTS

Dentist	Mean Periodontal Disease Rate	Standard Error of Mean	Dentist	Mean Periodontal Disease Rate	Standard Error of Mean
A. Chief of service....	48	6.8	E. Staff dentist.....	44	7.0
B. Periodontist.....	49	6.7	F. Staff dentist.....	40	7.2
C. Staff oral surgeon..	45	7.4	G. Dental intern	45	7.2
D. Staff dentist.....	45	7.6			

these two extremes was so small that it could easily have occurred by chance (critical ratio = <1).

It was concluded that the criteria were sufficiently precise to permit reproducible results by different examiners and that this group of examiners was adequately calibrated to warrant examination of large numbers. In actual clinical practice most of the examinations were completed by two examiners, although five additional dentists also contributed to the total of persons examined.

Classification of diseases.—It is never possible to state with certainty that there is no disease of any kind present in any individual, and in any population a substantial proportion of people is affected by undiscovered disease. At best, when there is no detectable evidence of disease, people may be considered relatively healthy. However, in a hospitalized population the probability of undetected disease is greatly minimized, and patients can be classified with greater confidence into those who are relatively healthy and those who are known to have specific diseases. It can be safely stated that the hospitalized patients of this study in whom *no* significant disease was found were in as good or better health than any similar unselected group from the general population. By the same token, confirmed diagnoses are quite likely to be accurate when determined in a modern general medical and surgical hospital.

In this study the most significant medical diagnosis was determined by one indi-

vidual (H. C. S.) after a review of the medical record of each patient. The most significant generalized disease was not always the same as the immediate reason for hospitalization. For example, a diabetic patient admitted to the hospital for elective surgery was classified in this study under the diagnosis of diabetes, for diabetes was the disease most likely to produce metabolic disturbance of greater severity. The influence of one systemic disease upon another is so complex that the assessment of a primary role was often impossible. The protocol provided that when a patient had two or more diagnoses of potentially equal generalized significance, as, for example, arteriosclerosis and diabetes, the patient was classified according to the disease given primary status by the physician in his listing of final diagnoses.

Although the original objective was to classify diseases according to specific medical entities, many diseases occurred so infrequently that they had to be broadly classified into non-specific systemic groups for effective statistical analysis. Patients hospitalized for minor somatic derangements, in whom no other significant diagnosis could be established, were grouped into broad classes of relatively healthy patients. Among these were those admitted for elective surgery, orthopedic disorders, traumatic injuries, and minor acute infections. In many of these patients, no other significant disease could be found after a thorough physical examination. It was reasonable to conclude that this group was at least as healthy as, and probably in better health than, the over-all population of white male veterans from which this sample was drawn. These patients provided a basis for comparison of periodontal disease prevalence as did the entire sample.

The average periodontal disease rates (PDR) and their standard errors were calculated for each subgroup, and comparisons were made within the framework of each decade of age (Table 2).

RESULTS

Tooth population.—The residual tooth population decreased with aging at a rapid rate. The number of teeth remaining in the mouth fell from an average of 27 teeth per patient at ages twenty to twenty-nine to 8 teeth per patient at ages sixty to sixty-nine (Table 3; Fig. 1). Although edentulousness was relatively rare in the youngest group, 43 per cent of patients were edentulous by the age of sixty to sixty-nine (Table 4).

Periodontal disease prevalence.—Since periodontal disease is rarely self-healing but tends to become progressively destructive, the age-specific prevalence rates assumed the form of a cumulative curve (Fig. 2), with the points of inflection at ages twenty-five and fifty-five. Between these ages periodontal disease increased almost in a straight line at the rate of about 1.8 per cent of remaining teeth per year.

Periodontal disease and generalized diseases.—The prevalence of periodontal disease was significantly higher among patients with relatively severe diabetes at all ages, and the differences were most marked in the younger age groups (Fig. 3). Patients with liver cirrhosis were also more extensively affected by periodontal disease. Patients with gastrointestinal disorders of all kinds and with arteriosclerosis seemed to have a somewhat higher periodontal disease rate, but the differences from normal were not statistically significant.

Patients with primary diseases of malignant neoplasms, neurological disorders, tuber-

TABLE 2

AVERAGE PERIODONTAL DISEASE RATE BY AGES AND PRIMARY DISEASE IN 3,994 DENTULOUS PATIENTS

PRIMARY DISEASES	TOTAL PATIENTS ALL AGES	AGES 20–29			AGES 30–39			AGES 40–49			AGES 50–59			AGES 60–69		
		No.	Mean	S.E.$_m$	No.	Mean	S.E.$_m$	No.	Mean	S.E.$_m$	No.	Mean	S.E.$_m$	No.	Mean	S.E.$_m$
All diseases	3,994	788	7.9	0.8	1254	26.1	1.0	866	49.0	1.4	411	68.2	2.0	675	71.8	1.4
1. Arteriosclerosis	155	1	.....	.....	12	17.5	8.4	17	51.5	11.0	33	78.3	6.2	92	81.7	3.2
2. Cardiac	121	9	.....	.....	23	32.0	7.9	37	47.4	7.0	18	69.1	11.1	34	67.6	7.0
3. Dermatoses	212	44	5.7	2.2	78	24.0	3.6	49	54.1	5.7	21	64.5	8.5	20	72.5	8.2
4. Diabetes	100	12	39.6	12.0	26	53.7	7.2	24	64.0	8.2	17	76.2	8.8	21	81.9	7.4
5. Elective surgery	902	260	7.9	1.3	270	25.0	2.2	181	44.2	3.0	64	62.7	5.3	127	68.2	3.4
6. Gastrointestinal	421	62	15.0	4.0	155	30.4	3.1	111	56.4	3.8	37	78.0	6.1	56	68.0	5.2
7. Genitourinary	49	10	.....	.....	11	34.5	11.6	8	.....	.....	6	.....	.....	14	73.2	11.6
8. Hemic-lymphatic	42	8	.....	.....	8	.....	.....	4	.....	.....	2	.....	.....	20	62.2	9.5
9. Infections (acute)	122	32	4.2	1.7	42	35.9	5.2	24	53.1	7.7	13	68.5	10.6	11	53.1	12.3
10. Kidney	81	13	6.2	4.2	33	12.6	3.8	17	44.4	9.7	9	.....	.....	9	.....	.....
11. Liver, cirrhosis	95	2	.....	.....	29	41.6	3.5	33	62.0	7.7	18	79.2	10.4	13	96.1	2.1
12. Liver, not cirrhosis	55	9	.....	.....	16	33.4	11.2	13	56.5	12.2	6	.....	.....	11	77.2	8.2
13. Malignant neoplasm	245	20	3.0	2.7	43	28.6	5.3	46	59.9	6.1	45	61.3	6.9	91	69.0	4.1
14. Musculoskeletal	32	7	.....	.....	10	.....	.....	10	.....	.....	2	.....	.....	3	.....	.....
15. Neurological	185	23	6.5	4.6	67	25.2	4.4	46	45.5	6.6	18	87.1	5.8	31	74.5	7.1
16. Orthopedic	147	44	2.8	1.2	49	25.2	5.2	28	41.8	6.8	15	66.0	15.7	17	74.4	8.2
17. Psychoneurotic	282	77	6.1	1.4	130	17.3	2.6	61	39.1	4.6	11	42.2	14.3	3	.....	.....
18. Respiratory	124	17	4.4	2.5	32	26.4	6.4	26	60.4	7.9	16	85.9	7.3	33	58.0	7.6
19. Traumatic	28	10	.....	.....	11	11.4	9.1	4	.....	.....	1	.....	.....	2	.....	.....
20. Tuberculosis	155	47	6.8	2.8	59	19.4	4.3	29	49.8	8.2	11	62.7	20.1	9	.....	.....
21. Others	435	81	7.8	2.7	150	25.7	2.9	98	46.9	4.2	48	61.7	5.4	58	72.3	4.9

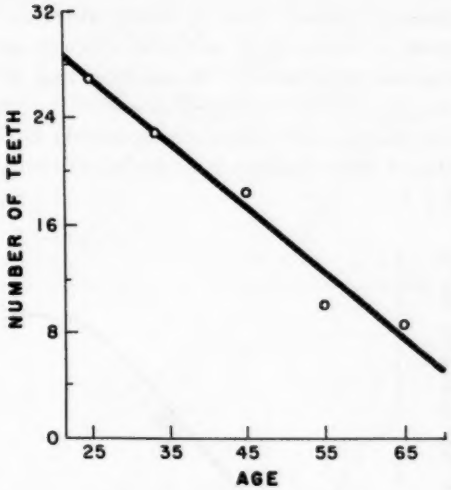

FIG. 1.—Average number of teeth present with aging in 4,832 hospitalized veterans

TABLE 3

AVERAGE NUMBER OF TEETH PRESENT BY AGE GROUP

Age	Patients Examined	Mean	S.D.	$S.E._m$
20–29	794	26.9	5.3	0.19
30–39	1,279	22.8	6.2	0.17
40–49	968	18.2	7.5	0.24
50–59	599	9.9	10.7	0.43
60–69	1,192	8.3	9.8	0.31
Total	4,832			

TABLE 4

HOSPITALIZED PATIENTS EXAMINED FROM JULY 1, 1957, THROUGH DECEMBER 31, 1958, DISTRIBUTED BY AGE GROUP AND EDENTULOUSNESS

	Age Group					
	All Ages	20–29	30–39	40–49	50–59	60–69
Total examined	4,832	794	1,279	968	599	1,192
Number dentulous*	3,994	788	1,254	866	411	675
Number edentulous	838	6	25	102	188	517
Per cent edentulous	17.3	0.7	1.9	10.5	31.3	43.3

* One or more teeth present.

culosis, respiratory diseases, cardiac diseases, kidney diseases, liver diseases (other than cirrhosis), orthopedic, dermatological, and other diseases demonstrated no significant differences in prevalence of periodontal disease from that of the group as a whole. Patients treated for elective surgery, orthopedic disorders, and traumatic injuries and those who had no other disease were considered relatively healthy. The age-specific periodontal disease rates of these patients were not significantly different from those of the total sample (Fig. 4).

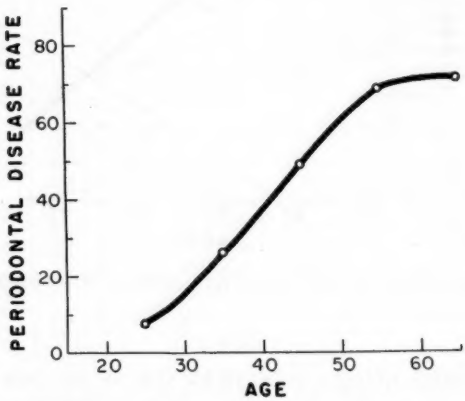

FIG. 2.—Prevalence of periodontal disease in 3,994 hospitalized veterans

DISCUSSION

Tooth loss.—The progressive loss of teeth with aging is the historical experience of mankind, and tooth loss in this sample of urban veterans is probably no greater than that of the general population. Although their generation did not have the advantages of fluoridation, children's dentistry, or periodontal care as we know it today, most veterans received some dental care while in the military services, and it was a rare exception who showed no evidence of previous dental care. Nevertheless, the increase in tooth mortality was extremely rapid.

Influence of generalized disease on periodontal status.—Clinical observation supports the opinion that periodontal tissues exposed to local traumatic influences tend to be less resistant in persons affected by systemic diseases. The consensus of most observers[18-25] was expressed at the Workshop on Periodontal Disease held at the University of Michigan,[26] as follows: "In every individual, systemic factors influence the initiation and the course of periodontal disease and the maintenance of periodontal health." Among disease entities specifically cited in this regard were blood dyscrasias,[27, 28] endocrine dysfunctions,[29] diabetes,[30, 31] nutritional deficiencies,[32, 33] and several others.[34-36]

The special circumstances concerning each of the disease groups of this study are worthy of mention since they may account for some of the findings which do not conform with those expected.

Patients with a primary diagnosis of diabetes were generally hospitalized because their disease was relatively uncontrolled and ketosis was not uncommon. Since the

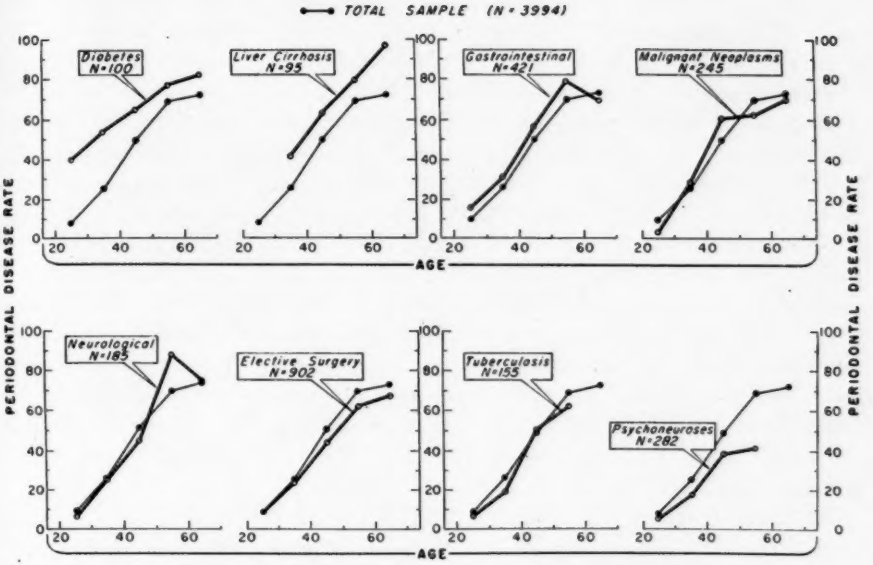

FIG. 3.—Average periodontal disease rate by age and primary disease

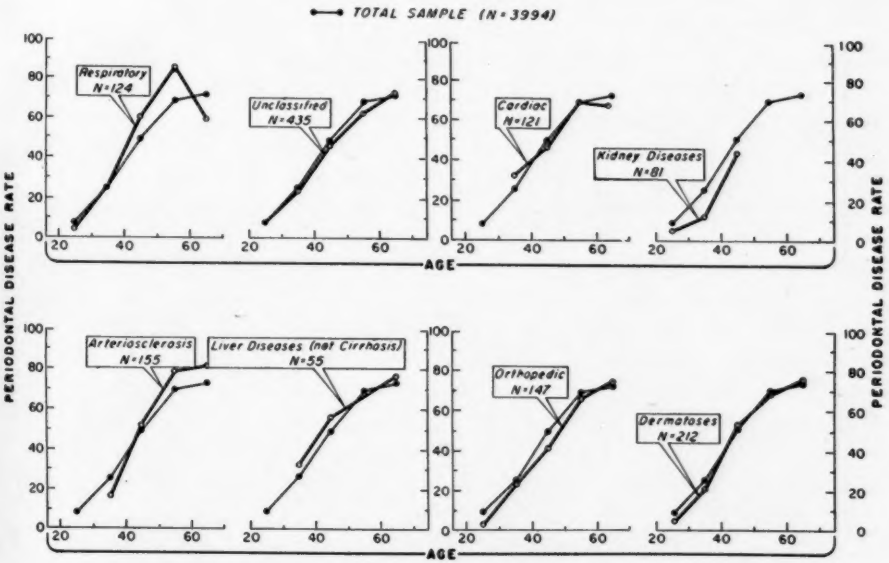

FIG. 4.—Average periodontal disease rate by age and primary disease

response to healing in uncontrolled diabetes is markedly delayed and circulatory impairment is quite common,[37] periodontal healing can be expected to be retarded. However, it is possible that patients with controlled diabetes may not demonstrate excessive periodontal disease.

Most of the 95 patients in the group with liver cirrhosis were chronic alcoholics of long standing. Their nutritional status, as well as their oral hygiene, was usually poor, but the dysfunction of the liver itself may have been a contributory factor through interference with normal protein metabolism.

The patients with gastrointestinal diseases did not represent a single disease entity. For the most part, the 421 patients in this grouping suffered from gastric or duodenal ulcers, and many had undergone subtotal gastrectomy or other gastrointestinal surgery. Those with confirmed neoplasms of gastrointestinal origin were not included but were classified under "malignant neoplasms." Nutritional deficiencies were common in these patients, and their medical histories usually revealed long-standing gastrointestinal disorders, frequent vomiting, limited diets, and poor food intake. The impaired nutritional absorption may have contributed to the somewhat greater prevalence of periodontal disease.

The types of malignant neoplasms in the 245 patients in this class were considerably different at different ages. The younger patients were more often those with lymphoma, leukemia, and a few sarcomas. The middle ages tended toward the squamous-cell, adenocarcinoma, melanoma, Hodgkins, and reticulum-cell sarcomas. Most of the relatively slow-growing basal-cell carcinomas were in the older age groups.

In the younger patients with neoplastic diseases the prevalence of periodontal disease was much the same as for the whole sample. There seemed to be a somewhat higher* prevalence of periodontal disease at ages forty to forty-nine. It is possible that this may reflect the greater generalized stress produced by the rapidly growing types of neoplasms compared with that of the slower-growing neoplasms noted in the youngest and oldest groups. Further study of this relationship would seem to be indicated.

Patients with neurological disorders were usually hospitalized for long periods, during which they received continuous dental care. However, those who were unable to care for their own hygiene seemed to develop periodontal disease regardless of frequent dental debridement and clinical care. The youngest groups of neurological patients were made up primarily of persons with residuals of traumatic injury, cranial tumors, infantile paralysis, and congenital dystrophies. The neurological manifestations of the patients over fifty years of age were, for the most part, the result of cerebral thrombosis or degeneration. The extent of periodontal disease in patients with neurological disorders was not markedly different from that of the total sample except for an increase in the ages of fifty to fifty-nine. These were the patients least able to care for themselves.

Periodontal disease rates among the 902 patients admitted for elective surgery with no other significant diagnosis were almost identical with that for the whole sample.

All the 155 patients with tuberculosis not only received complete and continuous dental care while hospitalized, but they were usually careful about their oral hygiene. Many of these patients had been ill for some time, and severe pulmonary tuberculosis

* Not statistically significant.

was quite common. Their periodontal status was very similar to that of the sample as a whole.

The 282 patients admitted for psychiatric treatment were those in whom a reasonably rapid improvement could be expected, and they were, for the most part, on an open ward. Few remained in the hospital for more than 6 months, and many were discharged in a shorter time. These patients seemed to have somewhat less periodontal disease than the sample as a whole. This difference may have been due to chance, but it was our impression that these patients represented a fairly intelligent group who were often more meticulous about their oral hygiene than most non-psychiatric patients.

The youngest and the oldest patients with respiratory diseases were mostly admitted for acute lung infections. Patients in the middle ages included many with chronic lung disease and asthma who were likely to be mouth-breathers. These latter groups seemed to have more periodontal disease than the group as a whole, but the differences were not statistically significant.

The 121 patients with cardiac diseases represented diagnoses of coronary thrombosis, congenital heart disease, rheumatic heart disease, cardiac insufficiency, and others. Those with known arteriosclerotic heart disease were not included in the cardiac diseases but were classified under an arteriosclerotic diagnosis. No increase of periodontal disease could be detected in this group.

The 81 patients who had kidney diseases were relatively young individuals. The diseases affecting the kidneys were quite dissimilar in their etiology and clinical consequences, and the small numbers in each group permitted no conclusions regarding periodontal status.

There were 155 patients with arteriosclerosis as the most important medical diagnosis. Only 13 were under forty years of age, so that no conclusions could be drawn regarding the younger patients, but those over the age of forty demonstrated somewhat greater periodontal disease prevalence than was expected. Histologic studies have shown that vascular degeneration is a common finding in the periodontal tissues[37] of these patients. It seems probable that the reduced blood supply to the periodontal area may have been a significant factor.

SUMMARY

A complete physical examination was provided for a sample of 4,832 hospitalized white male veterans. The oral examination included full-mouth radiograms and careful clinical evaluation of the periodontal structures. Each patient was classified according to the proportion of teeth affected by periodontal disease (PDR) and the disease entity or systemic disease of greatest generalized significance.

There were 838 edentulous patients. The residual tooth population in the remaining 3,994 persons declined rapidly with aging, from an average of 26.9 teeth per person at ages twenty to twenty-nine to 8.8 teeth per person at ages sixty to sixty-nine.

Eight per cent of teeth were affected by periodontal disease to the twenty-fifth year of age. Thereafter, periodontal disease increased rapidly at a steady rate of 1.8 per cent of remaining teeth per year until the age of fifty-five years. By the forty-fifth year, half of all remaining teeth were affected, and, at fifty-five, over 70 per cent of teeth

were affected. Periodontal tissues which had resisted disease up to the age of fifty-five tended to maintain this resistance with increasing age.

The influence of generalized diseases upon periodontal health was not remarkable except in patients with relatively severe diabetes and in those with cirrhosis of the liver. Periodontal disease was also higher, but not significantly, in patients with arteriosclerosis and gastrointestinal disorders.

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Correlation between Malocclusion, Oral Habits, and Socio-economic Level of Preschool Children

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In May, 1958, 491 preschool children received a dental examination as part of a complete physical evaluation for entering school in September. They ranged in age from three years eleven months to five years four months; the mean age was 4.65 years.

The dental examination had as its purpose (*a*) a dental educational experience for child and parent; (*b*) evaluation as to the needs of the child related to dental caries, periodontoclasia, and oral hygiene; and (*c*) an orthodontic appraisal of the dentition of the child. It is with the latter that this paper will be concerned.

EXPERIMENTAL METHODS

The examinations of the 491 children were completed by the same examiner, with the children's parents present. The orthodontic examination consisted of (*a*) observation of the opening and occluding of the dentition; (*b*) observation of the teeth in a static centric occlusion, to determine classification; (*c*) observation of the patient for any obvious habits; (*d*) examination of the patient's hands, lips, and tongue for evidence of habits; and (*e*) questioning of parent and child as to known habits which might influence the dental structures. Notation was also made of the presence or absence of spaces between the anterior teeth of the deciduous dentition.

Although Angle's classification of malocclusion[1] is admittedly not designed for the deciduous dentition and is incomplete in its description of all Class I malocclusions, it nevertheless was used in this study because of its efficiency in a survey of this type and its frequent use in the dental literature. The following are the classes referred to by Angle:[2, 3] Class I, normal mesiodistal relationship of the arches; Class II, distal relationship of the lower arch; Class III, mesial relationship of the lower arch. The position of the second deciduous molars and that of the maxillary canine were used to assess the arch relationship mesiodistally.

The following descriptions were used to denote tooth position when the maxillary and mandibular teeth were in contact and in centric occlusion: anterior open bite, premaxillary protrusion, deep overbite, end-on (edge-to-edge), and lingual posterior cross-bite.

Habit is defined as an action or condition which, by repetition, has become spontaneous.[4] The habits in this study were not differentiated but related to such actions or conditions as finger-sucking and similar hand pressures, fingernail biting, tongue habits, and lip or cheek habits.

A report[5] compiled by the United Community Services of Metropolitan Boston

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included a socio-economic classification of ninety Census tracts based on a method developed by E. Shevsky and W. Bell. This method produces a score of socio-economic status based on (*a*) median income for each Census tract; (*b*) percentage of persons twenty-five years old and over, in a given Census tract, who have completed 4 years of high school; and (*c*) percentage of total labor force in professional and managerial categories in each Census tract. It was on the basis of this method that the socio-economic status of a census tract was determined. Once this was established, the children were classified in high, middle, and low socio-economic groups according to the socio-economic status of the Census tract in which the school that the child attended was located.

RESULTS

Distribution of malocclusion[7] according to Angle's classification is shown in Table 1. It should be noted that 89.5 per cent of the cases fell into Class I. This compares favorably with 89 per cent found in a similar survey of 443 Brookline preschool children in 1951 by Cohen[7] and Cohen *et al.*[8] Angle's Class I was more heavily weighted in comparison with other studies carried on by earlier investigators in other groups.[1]

In relating malocclusion to the three socio-economic groups (Table 2), a chi-square test showed no significant relationship between socio-economic levels and malocclusion ($0.10 < P < 0.20$).

A great deal has been written about habits and their relation to malocclusion. Some

TABLE 1

DISTRIBUTION OF OCCLUSION BY NUMBER AND PER CENT OF 491 PRESCHOOL PUPILS BY ANGLE'S CLASSIFICATION

Occlusal Classification	No.	Per Cent
Total	491	100
No malocclusion	319	65.0
Class I malocclusion	154	31.3
Class II malocclusion	13	2.7
Class III malocclusion	5	1.0

TABLE 2

DISTRIBUTION OF OCCLUSION BY NUMBER AND PER CENT OF 491 PRESCHOOL PUPILS BY SOCIO-ECONOMIC STRATUM

Occlusal Classification	Socio-economic Stratum					
	High		Middle		Low	
	No.	Per Cent	No.	Per Cent	No.	Per Cent
Total	100	100	161	100	230	100
No malocclusion	60	60	105	65	154	67
Malocclusion	40	40	56	35	76	33

authorities believe that there is no relationship. Kaplan[9] has emphasized the need for further research in this area. English and Pearson[10] wrote of the "superstition that it [thumb-sucking] will deform the child's mouth." It should be noted that neither of the two afore-mentioned references was written by dentists. However, most workers in the field of dentistry, i.e., Lewis,[11] Johnson,[12] Swinehart,[13] and Lerner,[14] have conceded that there is a relation between a persistent habit and malocclusion.

Table 3 shows the distribution of malocclusions in children with and without habits. A chi-square test showed a significant relationship between habit and malocclusion ($0.003 < P < 0.005$). A statistically significant relation was also found in each socio-economic group (high $= 0.0005 > P$; middle $= 0.0005 > P$; low $= 0.0005 > P$).

There was a definite trend in the percentage distribution of habits as related to socio-economic levels (Table 4). A chi-square test showed a significant relation ($0.001 < P < 0.002$). Why habits should be significantly more frequent at higher socio-economic levels is a subject for future investigation.

As early as 1819, D. F. Delabarre included separation of deciduous teeth among the ten circumstances which would indicate that the second dentition would be "performed with ease." Hemley[15] has stated: "It is very common to find spaces or open contacts between deciduous teeth." Baume,[16] who concluded that spacing was con-

TABLE 3

DISTRIBUTION OF OCCLUSION BY NUMBER AND PER CENT OF 491 PRESCHOOL PUPILS BY HABITS

HABITS	TOTAL		OCCLUSAL CLASSIFICATION			
			Malocclusion		No Malocclusion	
	No.	Per Cent	No.	Per Cent	No.	Per Cent
Total	491	100	172	100	319	100
Habit	100	21.0	74	43.0	26	11.0
No habit	391	79.0	98	57.0	293	89.0

TABLE 4

DISTRIBUTION OF 491 PRESCHOOL PUPILS BY HABITS AS RELATED TO SOCIO-ECONOMIC STRATA

HABITS	TOTAL		SOCIO-ECONOMIC STRATUM					
			High		Middle		Low	
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
Total	491	100	100	100	161	100	230	100
Habit	100	21	28	28.0	42	26.0	30	13.0
No habit	391	79	72	72.0	119	74.0	200	87.0

genital, not developmental, found anterior spacing in 70 per cent of the cases studied. Contrary to these findings, this survey showed open spaces in 142 cases (30 per cent).

Many researchers have speculated that lack of spacing in the deciduous dentition gives rise to crowding and malocclusion of the permanent teeth. A succeeding survey in the group being reported on will lend some information in this area.

DISCUSSION

Based on the findings that higher socio-economic groups have significantly more oral habits than middle and low socio-economic groups and that habits are significantly associated with malocclusion, one should not be disturbed by the fact that there is no significant association of high socio-economic groups with malocclusion. Table 2 demonstrates that a trend in malocclusion from high to low socio-economic groups does exist, but, because a positive correlation was not demonstrated, it is not surprising to find the lack of a significant correlation between malocclusion and socio-economic groups. However, the significant relation of habit to malocclusion in each socio-economic group confirms the hypothesis that habit is related to malocclusion.

The second interesting finding was the high percentage of children with no anterior spacing in the deciduous dentition (71 per cent). Because approximately 75 per cent of the children included in this study will be re-examined in the fourth grade, the effect of spacing in the deciduous dentition on the occlusion and tooth position of the permanent dentition can be evaluated.

This study indicates that any future studies on spacing should contain a more definitive index as to degree of spacing and what measures determine that a space exists.

SUMMARY

An orthodontic survey was made on 491 perschool children who were grouped according to socio-economic level and Angle's classification of malocclusion.

No statistically significant relation was found to exist between socio-economic level and type of malocclusion. The relation between incidence of habits and of malocclusion was statistically significant. The positive correlation between incidence of habits and level of socio-economic strata was also significant. Contrary to prevailing information, 71 per cent of the children had no spaces between the anterior deciduous teeth.

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Dental Development of Children in a Siamese Village, Bang Chan, 1953

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The teeth of certain segments of the population were inspected as part of an investigation on the nutritional status of residents of Bang Chan, Thailand, under the auspices of the Cornell-Thailand Project. Dental development has been considered an indication of physical maturity in children.[1, 2] When birth dates are not known, dental age provides a convenient means of estimating chronological age when assessing the nutritional status of a young population.[3] To the student of nutrition, comparisons of dental development among populations with widely differing food habits are of interest. This report deals with the dental development of the children of primary-school age in Bang Chan, Thailand.

Although none of these children had any clear-cut deficiency disease, more than half of them had one or more possible signs of malnutrition.[4] The usual diet, in which rice provided over four-fifths of the calories and more than half the protein, appeared to be very low in certain nutrients, notably calcium, riboflavin, thiamine, vitamin A value, and probably linoleic acid.[5, 6] Comparison of the dental development of these children with those in the United States, where nutrient intake is more liberal and growth in height far exceeds that of Thai children, seemed worthwhile. Moreover, since the incidence of caries may be related to the length of time that teeth have been exposed to the external environment,[7] if caries incidence in two groups of children of the same age but of different racial or environmental backgrounds are to be compared, the number and kind of permanent teeth present at a given age should be comparable. Incidence of caries among school children in Bang Chan is described elsewhere.[8]

Two methods have been used to describe dental development: (1) determining the age of eruption of individual teeth, and (2) noting the number of teeth present at each age level. Cattell,[2] who was concerned primarily with dentition as a measure of maturity, used both methods. She studied data available for 11,656 American subjects classified according to ethnic origin, as of northern European, southern European,

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† The field study was carried out under the direction of this author, who received an award for advanced research from the United States Educational (Fulbright) Foundation in Thailand, 1952–53.

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Jewish, and Italian descent. The largest group examined was of northern European descent, including 3,863 boys and 3,826 girls. Data were grouped according to the age of the children at 6-month intervals and were tabulated separately for boys and girls. To determine the age of eruption of individual teeth, the percentage of children having a specific tooth erupted was calculated for each age level. A graph was made for each tooth. Ages at which 25, 50, and 75 per cent of the children had a given tooth erupted were denoted as the low, median, and upper quartiles of eruption. Cattell pointed out that the time of eruption of a given tooth may vary considerably in different children.

In a study designed to determine ages of eruption of permanent teeth, Klein, Palmer and Kramer[7] calculated for each year of age the percentages of boys and girls having specific teeth erupted. After plotting the data on arithmetic probability paper, they estimated the age at which 50 per cent would have a given tooth erupted.

Hurme[9] reviewed twenty-four studies on dental development and compiled the results with a view to establishing norms and standards for eruption of permanent teeth. Using those data which were complete and for which the sexes had been treated separately, he computed the mean and median ages of tooth emergence for boys and girls of western and northern European racial stock. Hurme found that mean ages of eruption were generally higher than median ages for incisors, while they were lower for first molars in both sexes. He noted that mean and median ages of tooth emergence should not be treated as corresponding terms.

Dahlberg and Menegaz-Bock[10] discussed the relative merits of the normal probability function as the basis for analysis of data on tooth emergence, and they advanced arguments to support its use. They also outlined the advantages of cross-sectional, as compared to longitudinal, studies of dental eruption.

A general idea of dental development of a group of children of the same age may be obtained by the second method, i.e., by determining the average number of permanent teeth present. Data on American,[7, 11] English,[12] and Japanese[13] children based on this method are available for comparison with observations on Thai children in Bang Chan.

EXPERIMENTAL METHODS

Subjects and examining personnel.—Of 233 children attending the primary school in Bang Chan, 5 were absent when dental examinations were made in February, 1953, and information on the age of 2 was inadequate. Hence 226 of these children were included in the study of dental development. In addition to the school children, whose teeth were examined by a dentist, 17 children in the same age group, but not attending Bang Chan primary school, were among the members of 31 families constituting the random sample of village families included in the study of nutritional status.[4] For 14 of these, the examining physician obtained the necessary information so that they could be included in the study of dental development. Thus records for 240 children were available for this study.

The age range among the school children was seven to fourteen years. Ordinarily they started to school at eight years and attended a minimum of 4 years. A few started earlier, and some who were thirteen or fourteen years old remained in the fourth grade because of delay in starting or failure. Thus the population examined

included almost all the village children between eight and twelve years old, and some of those younger or older.

Records.—The blank on which observations were recorded included spaces for identifying information: name, sex, residence (hamlet and house number), and grade in school. Each child's age to the nearest birthday was obtained from the birth date on the record of medical inspection and from the school records. If information from these two sources differed enough that the child's age at the nearest birthday was uncertain, the observations were not included in the study of dental development.

On the record, diagrams for deciduous and permanent teeth provided a space for each tooth, which was marked to indicate whether or not the tooth was erupting or erupted.

Classification of the data.—Data for children of each sex were grouped according to age at nearest birthday from seven to fourteen years. The difficulty in obtaining exact information on the ages of these children has been discussed in a previous publication.[4] Since the number of subjects at each age level was small and in a number of instances the age calculated from the birth date given at the time of medical inspection and that obtained from the school records did not agree exactly, grouping by 6-month intervals was not attempted.

TABLE 1

UPPER RIGHT SECOND MOLAR, GIRLS

Age (Years)	Eruption (Per Cent)	Age (Years)	Eruption (Per Cent)
10	15	12	45
11	70	13	82

A tooth was considered as erupted if any part of it was visible above the gum. At each age the total number of erupted teeth of a specific type was noted, and the percentages of specified kinds of permanent teeth erupted were calculated.

Computing median age of eruption.—The method used for estimating median ages of eruption of specific permanent teeth was similar to that used by Cattell[2] and by Hurme.[9] After calculating the percentage of boys and girls at each age having each specific tooth erupted, a graph was drawn for each kind of tooth for each sex. Cumulated percentages for each specified kind of tooth erupted were represented on the X-axis and age in years at 1-year intervals on the Y-axis. Median age of eruption was obtained by determining the point at which the age percentage eruption curve cut the 50 per cent perpendicular.

In most cases the curve was fairly regular, percentage eruption for a given tooth increasing with increasing age. Exceptions occurred in only 2 of the 56 cases (i.e., 28 teeth, both sexes), namely, the upper right second molar of girls and the lower right canine of boys. Figures for the former illustrate the problem (Table 1).

Such irregularities are to be expected in a cross-sectional study in which the number of children in each group is not large and the ages approximate. By considering the estimated age of eruption for corresponding teeth—in this case the upper left and the lower right and left second molars in girls—an appropriate choice between the two

possible figures could be made in each case. In the instance cited, the figure 70 per cent for age eleven was omitted from the series.

RESULTS AND DISCUSSION

Median ages of eruption of permanent teeth.—The median age of eruption for each type of permanent tooth, for boys and girls, is shown in Table 2. Differences in age of eruption of corresponding teeth on the right and left sides were slight and inconsistent, as has been noted in the majority of studies of tooth emergence;[10] hence the figures given are averages for corresponding teeth on the two sides. By the age of seven,

TABLE 2

MEDIAN AGES OF ERUPTION OF PERMANENT TEETH IN CHILDREN* OF BANG CHAN

Tooth	Jaw	Age (Years)	
		Boys Average of 2 Sides	Girls Average of 2 Sides
Central incisor	Maxillary	8.1	7.8
	Mandibular†	7.0–	7.0–
Lateral incisors	Maxillary	9.1	8.8
	Mandibular	8.2	7.6
Canine	Maxillary	11.5	10.9
	Mandibular	11.3	9.9
First bicuspid	Maxillary	10.5	9.8
	Mandibular	11.1	10.4
Second bicuspid	Maxillary	11.9	11.6
	Mandibular	11.8	11.5
First molar	Maxillary†	7.0–	7.0–
	Mandibular†	7.0–	7.0–
Second molar	Maxillary	12.2	12.1
	Mandibular	11.7	11.6

* For age-sex distribution see Table 3.

† More than 50 per cent of 7-year-old children had the tooth erupted; therefore age of eruption is stated as less than 7.0.

almost all the lower first molars and more than half the upper first molars and lower central incisors were erupted; hence age of eruption is given as less than seven. Six of the children examined in age groups eleven to thirteen had one or two of the third molars. As noted by observers in other areas, the median age at eruption for each permanent tooth was less for girls than for boys. Differences were marked in some instances—for example, the upper canine and first bicuspids—and slight in others, as in the second molars.

With one exception in both sexes, the order of eruption for each type of tooth was as follows: First molar, central incisor, lateral incisor, first bicuspid, canine, second bicuspid, and second molar. In girls the mandibular canine teeth erupted before the first bicuspids. In their study of tooth emergence in Pima Indians, Dahlberg and

Menegaz-Bock[10] found the order to be different in the maxilla but the same in the mandible for boys and girls. They also found the order to be different in the upper and lower jaws. These authors[10] subjected their data to probit analysis and commented that, among cross-sectional data, only those analyzed by similar methods should be compared. As they state, however, this does not preclude some value from informal comparison between miscellaneous groups. In the present study, as in that on Pima Indians,[10] all mandibular teeth except the first bicuspids were found to erupt before their maxillary counterparts.

The estimated median age of eruption for each pair of permanent teeth, i.e., the average of the values for corresponding teeth on the right and left sides of the same jaw, was compared with figures for children in the United States[7] and in India.[14] Recorded ages of some of the children in Bang Chan and in India were approximate,

TABLE 3

AVERAGE NUMBER OF TEETH: DECIDUOUS, PERMANENT, AND TOTAL, AT EACH AGE, IN CHILDREN 7 TO 14 YEARS OF AGE, BANG CHAN

Age (Years)	Boys				Girls			
	No. Examined	Average No. Teeth			No. Examined	Average No. Teeth		
		Dec.	Perm.	Total		Dec.	Perm.	Total
7.....	5	15.0	6.4	21.4	8	17.3	5.5	22.8
8.....	18	14.9	7.8	22.7	16	14.5	9.0	23.5
9.....	9	12.4	10.6	23.0	8	12.0	10.8	22.8
10.....	23	9.5	14.8	24.3	13	6.3	17.6	23.9
11*.....	27	6.8	17.9	24.7	24	4.6	19.8	24.4
12*.....	23	4.2	21.9	26.1	20	3.0	22.6	25.6
13*.....	17	0.5	26.3	26.8	22	0.7	27.4	28.1
14.....	3	0.0	28.0	28.0	4	0.0	28.0	28.0

* Six children in the age groups 11–13 had one or more third molars erupted.

and treatment of the data was not identical for the three studies; however, no marked or consistent differences were noted. For example, age of eruption for lateral incisors and second bicuspids appeared to be somewhat greater for children in Bang Chan than in the United States; the reverse was true for second molars and upper canine teeth. Our sample, however, was small, and the observations apply to the particular population studied, not to Thai children in general. Shourie,[14] who studied a larger group of Indian children in Madras and Lahore, stated that estimates seemed to indicate that in India the eruption of teeth takes place somewhat earlier than in the United States. He mentioned, however, that ages were approximate for many of the Indian children, as one could not rely on birth records or on the average person's statement of age. Voors,[3] who recalculated data from the literature, confirmed the statement of Hurme[9] that there is a real difference between population samples in mean eruption time for permanent teeth, and he commented that lower socio-economic classes appear to have accelerated dentition. Dahlberg and Menegaz-Bock[19] present some evidence that a complex of environmental and hereditary factors may be responsible for relatively late eruption of Pima Indian anterior teeth and the relatively early

eruption of Pima Indian posterior teeth when compared with teeth emergence in English children.

Average number of teeth per child at each age.—In Table 3 the average number of both permanent and deciduous teeth per child, at each age, is shown for boys and girls in Bang Chan. Between ages eight and thirteen, girls in Bang Chan had more permanent teeth than did boys. This finding is in harmony with observations in other areas. The average number of permanent teeth at each age for boys and girls in Bang Chan was compared with corresponding figures reported by investigators in the United States,[2, 11] England,[12] and Japan.[13] Most fourteen-year-old children in all these areas had all permanent teeth except the third molars. At younger ages, dentition of Thai children in Bang Chan was closer to that of Japanese children studied by Sutow[13] than to that of American or English children; but in none of the groups did dentition seem to be either consistently accelerated or retarded. Differences in caries rates between the children of Bang Chan and these other populations cannot therefore be attributed to differences in the length of exposure to external environment.

SUMMARY

The dental development of children aged seven to fourteen years in Bang Chan, Thailand, is described. Two hundred and forty children, 125 boys and 115 girls, were examined.

The median age of eruption is given for each type of permanent tooth for boys and girls. As noted in other areas, the median age of eruption for each permanent tooth was less for girls than for boys. Between ages eight and thirteen, girls in Bang Chan had more permanent teeth than did boys. At age fourteen, all children had all permanent teeth except the third molars, and 6 of those examined in age groups eleven to thirteen had one or two of the third molars.

Age of eruption of individual permanent teeth and total number of permanent teeth present at a given age in each sex were compared with corresponding data available for children in other parts of the world. No marked or consistent differences in dental development were noted between children in Bang Chan and elsewhere.

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Calcification. XXVI. Caries Susceptibility in Relation to Composition of Teeth and Diet

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More than a decade has passed since the thought was presented that there is an inverse relationship between dietary phosphate and caries susceptibility.[1] This was based in part on animal studies,[2] in which diets varying from high to low in phosphate content resulted in teeth varying from low to high in carbonate concentration, respectively. It was suggested that the low-carbonate teeth were less soluble in dilute acids and therefore more resistant to at least one type of caries, whereas high-carbonate teeth were more acid soluble and less caries-resistant. By 1952[3] this concept led to the first trial experiments on a series of cotton rats in which low-carbonate and high-carbonate teeth were produced by feeding high-phosphate and low-phosphate diets, respectively, prior to being placed on a cariogenic diet.[4] Two diets with greatly differing Ca/P ratios were selected in an attempt to highlight the effect on tooth carbonate composition. These experiments demonstrated that a high phosphate diet is cariostatic. The present report is an extension of these findings.

EXPERIMENTAL METHODS

Cotton rat colony.—The original breeding animals were obtained from the Public Health Institute of Lansing, Michigan. All animals in our stock colony were kept on a mixture of Pratt's pellets and Bill's stock diet.[5]

The conditions that proved successful in the breeding of these rats were as follows: a regimen of 12 hours of light and 12 hours of darkness, temperature-controlled at 78° F., a light-proof compartment available to the animals within the cage into which they can retire when disturbed, no bedding material, and daily handling as an aid in taming. Prior to the introduction of this last condition, the rats would injure themselves by jumping about wildly upon slight provocation.

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A preliminary report of part of this work was presented before (1) the Division of Biological Chemistry at the 121st meeting of the American Chemical Society, Milwaukee, Wisconsin, March 30–April 3, 1952, and (2) the Division of Biological Chemistry at the 124th meeting of the American Chemical Society, Chicago, Illinois, September 6–11, 1953.

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Animal regimen.—The experiments utilized 189 cotton rats which were distributed among the various experimental groups in such a way as to provide comparable hereditary backgrounds and comparable ratios of males to females. The experimental work extended over a 5-year interval from 1949 to 1954. In view of this extended collection of data and certain changing background influences, the results have been evaluated separately for the 1949–52 period and for the 1953–54 period. Between 1949 and 1952 the animals were wild and could be handled only with difficulty, whereas during 1953 and 1954 the animals were relatively tame and easier to handle. This treatment of the data permitted the best comparative evaluation of the experimental findings in a study conducted over such a lengthy period.

At 16 days of age—the normal weaning time for this species—the cotton rats were weaned and placed on either the low-phosphate–high-calcium diet (402) or the high-phosphate–low-calcium diet (403) for 4 weeks. Both these diets have a low ability to initiate and maintain carious lesions in the cotton rat through oral environmental influences. During the following period of 12–14 weeks, the corresponding cariogenic D and E diets, which contained similar calcium-to-phosphate levels (Table 1), were given. It was found necessary to incorporate sufficient calcium carbonate to produce a 0.1 per cent calcium level in the two high-phosphate–low-calcium diets, to prevent tetany.

It should be noted that, once mineralization has been completed in the molars, exchange with body fluids takes place to a limited degree only. This is in contrast with the continually growing incisors and with the bones, which undergo remodeling even after growth has ceased. At 16 days of age the crowns of the first molars of the cotton rat were almost completely mineralized, and the crowns of the second molars were partially mineralized, while the third molars developed, for the most part, during the experimental period. Hence only the compositions of the second and third molars would be influenced materially by the experimental diets.

At the end of the experimental periods, the animals were sacrificed by decapitation. The heads were immediately fixed in 95 per cent ethanol and left there for 48 hours. The jaws were stripped of skin and flesh and stored in fresh ethanol preparatory for observation. The number and extent of carious lesions were evaluated and recorded by the method of Shaw *et al.*[4, 6] The statistical significance of the results was evaluated by the methods outlined by Fisher.[7]

A weight record (Table 2) was kept of 94 animals to include the entire experimental period of 4 weeks on the preliminary diet followed by 12–14 weeks on the cariogenic diet.

Analytical studies of bone, tooth, and blood composition.—Analysis of 120 cotton rats to determine differences in composition of blood, bone, and teeth following 4 weeks on diets similar to the preliminary high- and low-phosphate diets (Table 1) used in this investigation have been previously reported.[8] Serum calcium and phosphorus were determined in 133 animals following the cariogenic dietary period (Table 3). The bone CO_3:PO_4 ratios of a series of 60 animals maintained 4 weeks on the preliminary diets followed by 12–14 weeks on the corresponding cariogenic diets (Table 4) were determined during each experimental period. The teeth of these animals were not analyzed because of changes due to the process of decay. The analytical methods used in this study have been previously described.[2, 9-12]

TABLE 1

COMPOSITION OF DIETS

Diets	Food Components	Grams	Mineral Components	Grams	Vitamins	Ca (Per Cent)	P (Per Cent)	Ca/P
Basal I	Yellow corn meal (degerminated) Wheat gluten Liver extract 1:20	75	Salt mixture*	2.36	Vitamin mixture,* 235 mg.	0.0286	0.118	0.242
Basal II	Sucrose Casein Corn oil Liver extract	67 24 5 0.5	Salt mixture*	2.36	Vitamin mixture,* 240 mg.	0.036	0.093	0.387
402 (low P, high Ca)	Basal diet I	97	$CaCO_3$	3.0	.....	1.23	0.118	10.4
403 (high P, low Ca)	Basal diet I	96	Na_2HPO_4 $CaCO_3$	3.19 0.250	.....	0.11	0.818	0.134
D (cariogenic low P, high Ca)	Basal diet II	98.47	$CaCO_3$	2.94	0.1 ml. haliver oil per kilo diet†	1.21	0.09	13.3
E (cariogenic, high P, low Ca)	Basal diet II	96	Na_2HPO_4 $CaCO_3$	3.48 0.250	0.1 ml. haliver oil per kilo diet†	0.103	0.858	0.120

* Composition of salt mixture and vitamin mixture:

SALT MIXTURE		VITAMIN MIXTURE	
Components	Grams	Components	Grams
NaCl	0.84	Thiamine	0.25
KCl	0.72	Riboflavin	0.30
$MgSO_4 \cdot 7H_2O$	0.53	Nicotinic acid	2.50
$Fe(C_6H_5O_7) \cdot 6H_2O$	0.15	Pyridoxine	0.25
$MnSO_4 \cdot 2H_2O$	0.03	Calcium pantothenate	2.00
ZnCl	0.01	Choline HCl	100.00
$CuSO_4 \cdot 5H_2O$	0.03	Inositol	100.00
KI	0.05	*p*-Amino benzoic acid	30.00

† Haliver oil is an Abbot #3532 product which contains 5,450 units of vitamin A and 900 units of vitamin D per 0.1 ml.

TABLE 2

DIET VERSUS WEIGHT CHANGE

Diets	Total Time on Diet*	No. of Animals	Average Weight Gain†	Average Weight Gain per Week‡
402 and D	14	21	73.4	5.2
403 and E	14	12	81.7	5.8
402 and D	16	15	85.2	5.3
403 and E	16	13	82.7	5.2
402 and D	18	7	100.9	5.6
403 and E	18	7	102.1	5.7
402 and D	20	11	94.9	4.7
403 and E	20	8	100.8	5.0

* The total time on experiment consisted of 4 weeks on either the preliminary non-cariogenic 402 or 403 diet followed by the cariogenic D or E diet, respectively.

† Pooled average of male and female weights.

‡ The corresponding figure for stock-diet animals during a corresponding time interval of 14–20 weeks was found to be 10.2 gm.

RESULTS

Weight changes.—The mean weight gain per week of 15 animals on the low-phosphate preliminary and cariogenic diets for a total period of 16 weeks was 5.3 gm. The corresponding figure for 13 animals on the high-phosphate diet was 5.2 gm., while for control animals on stock diet the mean gain was 10.2 gm. per week (Table 2). All averages refer to pooled groups divided to maintain equal ratios of males and females.

Composition of blood, bone, and teeth following the preliminary diet.—The CO_3:PO_4 ratios of bones and teeth and the serum calcium and phosphorus of animals prior to being placed on the cariogenic diets reflected the nutritionally induced production of high-carbonate and low-carbonate bones and teeth.[8] It should be noted that in the earlier work PO_4:$2CO_3$ ratios were given, whereas in the present study CO_3:PO_4 ratios were calculated, to visualize more readily the relative carbonate content.

Composition of blood and bone following the cariogenic diet.—As seen in Tables 3

TABLE 3

CO_3:PO_4 MOLAR RATIO IN BONE OF COTTON RATS ON CARIOGENIC DIET FOR 12–14 WEEKS*

DIETS	TIBIA		FEMUR	
	1949–52†	1953–54‡	1949–52†	1953–54‡
402 and D	0.156	0.222	0.164	0.222
403 and E	0.106	0.181	0.104	0.155

* All *P* values were found to be less than 0.05 in comparing the two dietary groups.

† Following the weaning period, 14 animals were placed on Diet 402 for 4 weeks followed by Diet D for 12–14 weeks and 9 animals on Diet 403 for 4 weeks followed by Diet E for 12–14 weeks.

‡ Following the weaning period, 15 animals were placed on Diet 402 for 4 weeks followed by Diet D for 12–14 weeks and 22 animals were placed on Diet 403 for 4 weeks followed by Diet E for 12–14 weeks.

TABLE 4

SERUM CA AND P LEVELS IN COTTON RATS ON CARIOGENIC DIET FOR 12–14 WEEKS

DIETS	CA (MG PER CENT)		P (MG PER CENT)	
	1949–52†	1953–54‡	1949–52†	1953–54‡
402 and D	13.1	10.3	2.6	6.1
403 and E	6.8	8.5	7.1	8.2

* All *P* values were less than 0.05 in comparing the two dietary groups.

† Following the weaning period, 57 animals were placed on Diet 402 for 4 weeks followed by Diet D for 12–14 weeks, and 39 animals were placed on Diet 403 for 4 weeks followed by Diet E for 12–14 weeks. During 1949–52 carbonate analyses were also performed, averaging 50.0 vol. per cent for the 402-D group and 52.9 vol. per cent for the 403-E group.

‡ Following the weaning period, 15 animals were placed on Diet 402 for 4 weeks, followed by Diet D for 12–14 weeks and 22 animals were placed on Diet 403 for 4 weeks followed by Diet E for 12–14 weeks.

and 4, analyses of calcium and phosphorus in serum and CO_3:PO_4 ratios in bone show that the high- and low-phosphate cariogenic diets maintained the composition differences established by the preliminary diet.[8] When the data for 1949–52 and 1953–54 were considered separately, the means of the CO_3:PO_4 ratios for the group of cotton rats fed the low-phosphorus—high-calcium diet were found to be significantly higher (P values less than 0.01) than the corresponding means for the group of cotton rats fed the high-phosphorus–low-calcium diet. However, in 1953–54, a sharp trend in the direction of higher CO_3:PO_4 ratios was noted for both dietary groups (Table 4). Another trend was noted in the direction of less extreme differences in serum calcium and phosphorus values of the two dietary groups during 1953–54. The rise in serum P from 2.6 in 1949–52 to 6.1 in 1953–54 was the most pronounced change (Table 3).

Influence of diet on caries susceptibility.—Statistical evaluation of both the number and the extent of carious lesions showed that the influence of diet on caries susceptibility was significant (Table 5). During 1949–52 the rats with the high-carbonate teeth averaged 11 lesions with an extent of 25.2^+, whereas the rats with the low-carbonate teeth averaged 5.3 lesions with an extent of 13.1^+. Parallel results were obtained during 1953–54; the rats with the high-carbonate teeth averaged 15.6 lesions with an extent of 44.9^+, and the rats with the low-carbonate teeth averaged 8.6 lesions with an extent of 22.9^+. Figure 1 illustrates the distribution of number and extent of carious lesions for 1949–52. A comparable distribution was observed during 1953–54.

DISCUSSION

It is evident from these studies that a high-phosphate diet is cariostatic as compared to a low-phosphate diet. As seen in Table 5, both the extent and the severity of caries were reduced to about half during the 1949–52 period, as well as during the 1953–54 period. Although the relative difference is about the same, the absolute severity is greater in both dietary groups in the later period. Factors such as genetic changes in the strain of cotton rat, brought about by inbreeding in our laboratories, as well as the tameness of the later animals induced by constant handling may have contributed to the differences in the severity of caries. Additional causes may have been accidental variations in other cariostatic factors in the diet (i.e., fluoride) or changes in the oral bacterial flora.

At the end of the caries-producing period, the diet, blood, and bone compositions were found to be related (Tables 3 and 4). The high-phosphate diet resulted in elevated serum phosphate and lowered bone carbonate. The composition of the teeth was not studied, since the caries process superimposes additional composition changes. For example, carbonate and citrate are probably dissolved preferentially from the tooth surface. In studies reported earlier, it was found that, after 4 weeks on the preliminary diet prior to the caries-producing regimen, the diet was related to changes in composition not only of bone but also of the enamel and dentin of the molars and incisors.[8] Since the changes in mineral composition of bone were still related to the diet after 12–14 weeks on the cariogenic diet, it may be inferred that similar changes in tooth composition would also have been found if the teeth had not been subjected to the process of caries.

Confirmation of the cariostatic effect of high-phosphate diets observed in this lab-

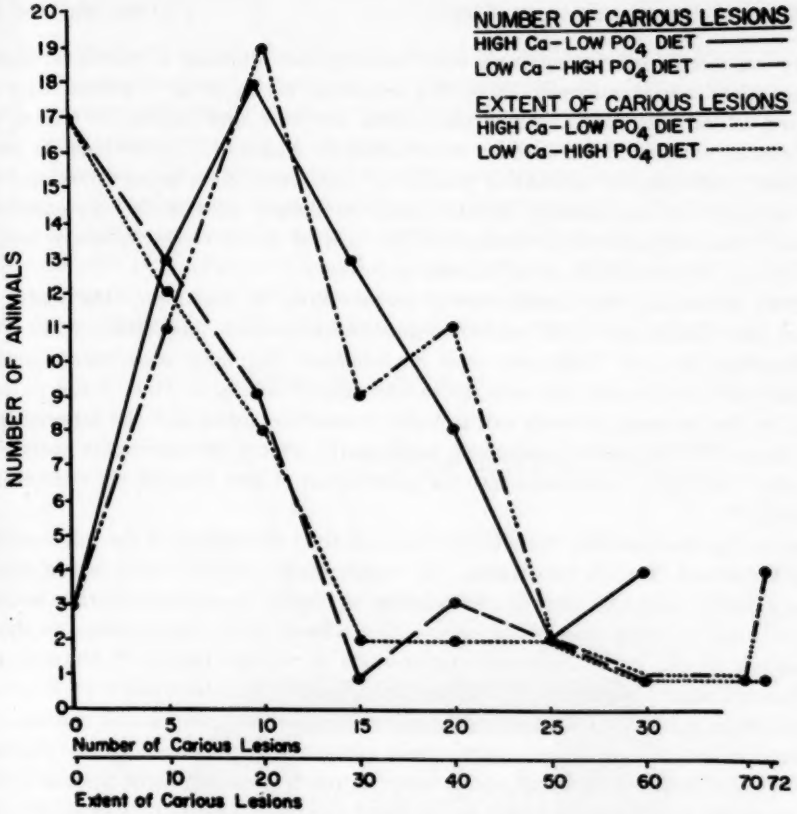
NUMBER OF CARIOUS LESIONS
HIGH Ca – LOW PO_4 DIET
LOW Ca – HIGH PO_4 DIET
EXTENT OF CARIOUS LESIONS
HIGH Ca – LOW PO_4 DIET
LOW Ca – HIGH PO_4 DIET
NUMBER OF ANIMALS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
0 5 10 15 20 25 30
Number of Carious Lesions
0 10 20 30 40 50 60 70 72
Extent of Carious Lesions

FIG. 1.—Distribution of number and extent of carious lesions during 1949–52

TABLE 5

CARIES SUSCEPTIBILITY AND HIGH PO_4 DIET IN THE COTTON RAT*

Diet	Period	No. of Animals	Number of Carious Lesions† (Mean Values)	Extent of Carious Lesions† (Mean Values)
Low P, High Ca 4 wk., +sucrose 12–14 wk.	1949–52	61	11.0	25.2†
	1953–54	30	15.6	44.9†
High P,‡ Low Ca 4 wk., +sucrose 12–14 wk.	1949–52	41	5.3	13.1†
	1953–54	38	8.6	22.9†
P	1949–52	.....	$\ll 0.01$	< 0.01
	1953–54	.....	< 0.005	< 0.005

* Age of animals at beginning of experiment was 16 days. Litters split between the two experimental diets. See Ref. 16.

† Caries evaluated by Professor James H. Shaw, Harvard School of Dental Medicine.

‡ Phosphate added as 3.2–3.5 per cent Na_2HPO_4.

oratory has been forthcoming from other investigators utilizing a variety of experimental animals and cariogenic diets. For example, Wynn *et al.*[13] worked with desalivated albino rats fed a sucrose diet, while McClure and Muller,[14, 15] using the same species, eliminated the need for desalivation by means of a lysine-deficient cereal diet. Nizel and Harris,[16] as well as Strålfors,[17] fed cereal diets to hamsters, and the latter worker also successfully initiated trials of dietary phosphate supplements in humans.[18] Such agreement is unusual in the field of caries research, where lack of experimental reproducibility is a frequent occurrence.

Factors influencing the composition of teeth.—Prior to eruption of the tooth, the internal body fluids and "local factors" influence its mineral composition. After eruption, however, the oral fluids also exert an influence. Sognnaes' experiments suggest "a liquid-solid relationship between saliva and enamel similar in kind, if not in magnitude, to that existing between extracellular connective tissue and the mesenchymal hard tissues."[19] The oral environment particularly affects the outermost surface of the tooth,[20] but there is evidence also for penetration of ions through the enamel into the dentin.[19]

Data are sparse regarding the effects of diet on the composition of the saliva, which can be influenced through two routes: (*a*) systemically, via the blood serum (internal body fluids), and (*b*) directly, by solution of dietary components during feeding. There is some evidence that blood composition affects saliva composition, as shown by Shannon *et al.*, for 17-hydroxycorticosteroids in parotid fluid.[21, 22] Shannon has also observed that in hyperparathyroidism (three cases) phosphate levels are low both in blood serum and in parotid fluid.[23] Moreover, there are indications that calcium and phosphate in salivary secretions, particularly phosphate, are influenced by the diet. For example, Clark[24] found that about twice as much phosphate was present in the saliva of sheep given a normal diet as in sheep receiving a phosphate-deficient diet. Buttner and Muhler[25] found an increase in salivary phosphate following oral administration of $CaHPO_4$. If phosphate is increased in the saliva and carbonate remains constant, the likely result may be a tooth surface lower in carbonate than in phosphate. There are no indications of the degree to which soluble phosphate (Na_2HPO_4 added to the diet) dissolves directly in the oral fluid, but during chewing one would expect considerable dissolution. Additional studies are needed to establish the systemic and oral effects of the diet on saliva composition and to elucidate the role of the saliva in caries production.

Caries hypothesis and the mineral composition of teeth.—The premise that led to the design of these experiments[26, 27] is given below in its most recently developed form. It was postulated that high-carbonate teeth are more soluble, since carbonate is preferentially dissolved.[28-33] This can be better understood if we examine the composition of the tooth mineral in relation to its structure (Fig. 2). In the present concept of the inorganic fraction of both bones and teeth it is considered that the mineral of both tissues is present in two discrete phases—a crystalline core of hydroxyapatite, surrounded by a tightly bound amorphous adsorption layer. According to Carlström,[34] the composition of the lattice unit cells comprising the crystalline core can vary from $Ca_6(PO_4)_6(OH)_2^{-8}$ to $Ca_{14}(PO_4)_6(OH)_2^{+8}$. Other constituents of the bone or tooth mineral, such as carbonate, citrate, fluoride, as well as other anions and cations, which are deposited in variable amounts, occur primarily in the amorphous adsorption layer.

The composition of the adsorption layer, therefore, would determine the initial solubility of the tooth mineral. Citrate and carbonate present in relatively large amounts would increase tooth solubility, while fluoride would decrease solubility.

The proportions of the layer of adsorbed ions and the hydration shell shown in Figure 2 are schematic. The carbonate portion of the adsorption layer would occupy 3–16 per cent of the volume occupied by the hydroxyapatite crystal. This volume was calculated by assuming that the specific gravities of calcium carbonate ($CaCO_3$) and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] are about the same, in which case 10 carbonate ions as calcium carbonate and 6 phosphate ions as hydroxyapatite occupy equal volumes. By multiplying the CO_2:PO_4 ratio (calculated from Table 1 of ref. 27) by 0.60

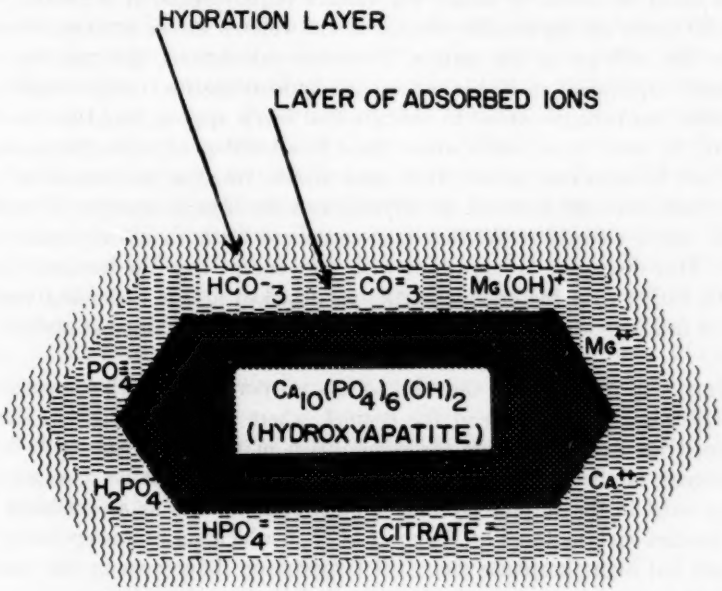

FIG. 2.—Bone of tooth crystal in body fluid (schematic diagram)

(6:10), the relative volume occupied by the surface carbonate compared to the apatite crystal volume is obtained. A second assumption is that 1 mole of absorbed calcium carbonate occupies the same volume as it would if it were an independent solid.

The actual volume occupied by the layer of adsorbed ions is probably greater than the calculated value, since this layer contains other ions, such as citrate, $HPO_4^=$, $Mg(OH)^+$, etc. In addition, the specific gravity of hydroxyapatite is slightly higher than that of calcium carbonate.

There is a tightly bound "hydration shell" surrounding and within the adsorption layer, containing 18 millimoles of water per 1 gram of hydroxyapatite, according to Myers' recent report.[34] This would be approximately 5.4 per cent of the volume occupied by the hydroxyapatite crystal.

If this concept is valid, then the point of attack in caries could depend on those crystal surfaces which happen to be higher in soluble components—in the case of the

present studies, carbonate. By the law of mass action these points would be more readily dissolved. Our calculations from earlier experiments[8] indicate that in high-carbonate teeth a volume equivalent to 7.9 per cent of the hydroxyapatite phase of the enamel is occupied by carbonate, while in low-carbonate teeth the carbonate volume corresponds to 4.6 per cent of the hydroxyapatite phase. This difference could be sufficient to bring about greater ease of acid attack. If, in the high-carbonate teeth, there are local areas still richer in carbonate than these over-all figures indicate, then at those sites the attack could be even easier. In this connection the maximum binding that can take place, according to Carlström,[35] is of considerable interest. In the case of a lattice unit cell whose composition is $Ca_{14}(PO_4)_6(OH)_2^{+8}$, four carbonate ions could be bound to satisfy the valence requirements. It is evident that their loss would cause an appreciable change in the volume of the crystals, where almost half the unit cells are at the surface. From our calculations, the maximal volume of carbonate is equivalent to 40 per cent of the hydroxyapatite crystal volume, although no evidence has been presented to indicate that levels approaching this have occurred in nature. At such focal points where there is a buildup of carbonate concentration, there would be increased sensitivity to acid attack, resulting in removal of carbonate and increased distance between the crystal and the organic matrix. It would follow from the above considerations that high-carbonate teeth should be more caries-susceptible. This discussion is consistent with the concept that the inorganic portion of the tooth, rather than the organic portion, is attacked first in caries but would be inconsistent in a hypothesis in which initiation of caries in the organic portion is postulated.

In terms of incipient caries, the most significant portion of tooth composition would be the crystals at the surface of the enamel, where Brudevold's group showed that the composition can differ from the composition of the deeper layers.[20, 36] Thus, in gross analysis of the enamel, composition differences may not be revealed, although they may exist in the surface layer. One must consider this as a possibility in interpreting studies such as those of Wynn *et al.*,[13] in which caries susceptibility was less in animals fed high-phosphate diets, yet composition differences in the enamel were not evident.

Oral effects of high-phosphate diets.—A change in the composition of saliva brought about by diets high in soluble phosphate can affect caries susceptibility in several ways. First, salivary phosphate may be increased via either systemic or direct oral effects. The elevated activity product $(A_{Ca}{}^{++})(A_{HPO_4}{}^{=})$ which would result would decrease dissolution of the tooth mineral, and, if the product were sufficiently high, it might actually be instrumental in remineralizing the partially dissolved surface of the enamel crystal. Second, by increasing salivary phosphate, the CO_3:PO_4 ratio, as well as the citrate:PO_4 ratio,[37] is decreased, decreasing the solubility of the tooth mineral. Third, phosphate is a powerful buffer, and increased salivary phosphate, particularly by direct solution in the saliva or adsorption to the tooth surface during chewing, would neutralize bacterial acids. A fourth possible way in which increased salivary phosphate may decrease caries susceptibility may be by alteration of the bacterial flora present in the mouth.

Evaluation of the proposed caries hypothesis.—The experiments derived from our caries hypothesis based on changes in tooth composition, led to the finding, confirmed

in several laboratories, that soluble phosphate added to the diet is cariostatic. Careful evaluation of the results, however, indicate that, in addition to affecting tooth composition, a direct effect of phosphate within the oral cavity, such as neutralization of acids and decreased solubility of tooth mineral, could also bring about the cariostatic result. We hope that future investigations will more closely define the mechanism through which the cariostatic effect of phosphate supplements to the diet is accomplished.

SUMMARY

Studies of caries susceptibility were undertaken to test the hypothesis that low-carbonate teeth are less caries-susceptible than high-carbonate teeth. Split litters of cotton rats were fed high-phosphate–low-calcium and low-phosphate–high-calcium diets for 4 weeks to produce low-carbonate and high-carbonate teeth. This was followed by high-sucrose cariogenic diets with similar calcium and phosphate content, respectively, for 12–14 weeks. At the end of the experimental period, serum calcium was lower, serum phosphate was higher, and the CO_3:PO_4 ratio of both femora and the tibias were lower in animals fed the high-phosphate–low-calcium regimens. These animals were significantly less susceptible to dental caries than were animals on the low-phosphate–high-calcium diet. Both the number and the extent of carious lesions were reduced to about half in animals fed the high-phosphate–low-calcium diet.

Although it has been confirmed in several laboratories under a variety of conditions that diets high in soluble phosphate are cariostatic, it is not possible at present to determine which of the following factors produced the cariostatic effect: (1) systemic effect on serum composition, influencing tooth composition and solubility; (2) systemic effect on saliva composition, influencing tooth composition and solubility; (3) direct oral effect due to solution of dietary phosphate in saliva, which may influence tooth composition and solubility and may neutralize bacterial acids, as well as interact in other ways with the bacterial flora.

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Effect of Naturally Occurring Selenium and Vanadium on Dental Caries

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The recognition of the caries-inhibiting effect of minute amounts of fluoride in the drinking water has stimulated interest in the possible effects of other trace elements or micronutrients on dental pathology.

A number of studies have indicated that selenium and vanadium may be present in teeth.[1-3] English[4] studied the effect of selenium dihydroxide on the teeth and jaws of a litter of dogs and found that large dosages of the selenium compound resulted in structural changes in the dentin and variations in size and shape of the mandibular condyles. Wheatcroft, English, and Schlack[5] administered sodium selenite intraperitoneally to rats and observed an increase in dental caries in those which received the toxic dose of 1.0 mg/kg of body weight. No increase in dental caries was observed by Muhler and Shafer[6] in their study on rats fed a cariogenic diet to which sodium selenite was added.

None of the investigators of selenium toxicity of livestock and domestic animals in seleniferous regions mentions any changes in the teeth of these animals.[7-10] However, one of the symptoms of selenosis observed in humans living in seleniferous areas was bad teeth,[11, 12] although the authors were not certain whether this could be attributed to selenium.

Hadjimarkos, Storvick, and Remmert[13] and Hadjimarkos and Bonhorst[14] studied the effect of selenium on the teeth of children in regions where selenium is not known to occur. They found a slight but significant direct relationship between the urinary concentration of selenium and susceptibility to dental caries.

Selenium is the only mineral element known that is present in food and forage crops in sufficient amounts to make them lethal to animals and perhaps toxic to man.[7] Smith and Westfall[11] and Smith, Franke, and Westfall[12] surveyed families living in seleniferous areas and reported that selenium was ingested in appreciable quantities. Injury to humans may arise from the consumption of seleniferous grains, vegetables, dairy products,[7] and eggs;[15] from fruit sprayed with insecticides contaminated with selenium;[16] from meat of contaminated livestock;[7, 11, 17, 18] from industrial hazards;[7, 19] and from pharmacological preparations.[20-22] Smith and Lillie[17] postulated that, in man, a daily intake of 1.0 mg. of selenium/kg of body weight was dangerous and that continuous ingestion of food selenium in quantities as low as 0.2 mg/kg of body weight was potentially harmful.

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As with selenium, controversial results on caries susceptibility also seem to have been obtained with vanadium. Geyer[23] has suggested that vanadium may replace part of the phosphorus ions of the hydroxyapatite of the tooth structure. In a study on hamsters maintained on a diet containing 0.04 and 0.08 mg. of vanadium pentoxide daily, he observed that vanadium had a caries-inhibiting effect. However, Hein and Wisotzky[24, 25] observed significant increases in caries incidence in hamsters given, instead of water, an aqueous solution of vanadium pentoxide at a concentration of 10 ppm. In studies on rats given vanadium pentoxide in concentrations of 10, 20, or 40 ppm in drinking water, Muhler[26] obtained results similar to those of Hein and Wisotzky. All animals in these studies were maintained on cariogenic diets.

Vanadium is considered to be one of the more abundant trace elements[27] and is widely spread in minute quantities. It occurs in algae and the blood of marine organisms,[7, 28, 29] which gives rise to the hypothesis concerning its presence in sedimentary rocks[7] and consequently in water. The foodstuffs which were analyzed spectrographically by Daniel and Hewston[30] did not contain vanadium. Although it is assumed that vanadium is an essential micronutrient for green plants,[29, 31] it is not known whether humans require this element.

The purpose of this study was to determine the possible effects of selenium and vanadium on dental tissues. No investigations prior to this report have been made on the effect of these trace elements on the teeth of subjects living in seleniferous areas. Wyoming was selected as the site for the study because it has extensive seleniferous areas and widespread vanadium deposits.

EXPERIMENTAL METHODS

Selection of areas.—Selection of the 15 areas was based on the geological distribution of selenium,[7] the occurrence of the element in vegetation,[7, 32] and the incidence of selenosis in livestock.[7] The control areas were comparable geographically and climatologically to the seleniferous,[33-36] but no selenium deposits, contaminated vegetation, or livestock which showed symptoms of selenosis had been discovered in the control areas.[37]

Selection of subjects.—The subjects studied were limited to white native-born-and-reared boys and girls from ten to eighteen years old. The wide age range was necessitated by the fact that many of the areas—arid and semiarid—were sparsely settled. The children were considered native-born-and-reared if they had been born in the study area and had not been absent from it for more than 2 months in any year. Age was defined to the last birthday. The water supply of the children was from the same source since birth. Children who had received topical applications of fluorides were not included. These specifications limited the number of available subjects to 456.

Oral examination.—Examinations were made and recorded by one dentist (G. T.), which limited variance to a minimum.[38] The oral area was dried with the aid of a chip blower. Mouth mirrors and explorers were used under good auxiliary light.

The symbols "DMFT"* for the permanent and "dmft"* for the primary teeth were used to designate caries experience. Third molars were excluded. A tooth was considered carious if the explorer caught in the enamel or dentin and if, upon thorough

* Decayed, missing, and filled teeth.

investigation, evidence of a lesion was detected. Observations on permanent and primary teeth included the following: (1) Number of carious teeth indicated for filling. If a tooth was both filled and decayed, it was considered as one DMF tooth. (2) Number of carious teeth indicated for extraction. If all surfaces of a tooth were completely destroyed by caries, it was considered as a tooth indicated for extraction and included in the symbol as a missing tooth. (3) Number of carious teeth extracted. Extracted teeth included in the symbol as missing teeth were assumed to have been lost because of extensive caries. In primary teeth this was confirmed through interview, age of the child, and presence or absence of the homologous tooth on the opposite side of the arch. When lost by accident, extracted for orthodontic reasons or because it was expected to exfoliate soon, a tooth was not listed as extracted. (4) Number of teeth filled. (5) Number of carious surfaces. Each posterior tooth was considered as a tooth with five surfaces and each anterior tooth as one with four. (6) Number of teeth unerupted. (7) Number of teeth exfoliated. (8) Incidence of enamel hypoplasia. (9) Incidence of dental fluorosis and severity of the defect. Dean's fluorosis index[39] was used. (10) Presence of gross malocclusion of the mixed and permanent dentition. No effort was made to count the number of malocclueded teeth. The following observations were made from examination of the hard and soft tissues of the mouth: absence of teeth, presence of supernumerary teeth, and ectopic eruption of teeth. Occlusion was observed in centric closure of the jaws.

Schour and Massler's quantitative measure of the incidence of gingivitis, the PMA index,[40] was used, and all maxillary and mandibular gingival units, consisting of papillary (*P*), marginal (*M*), and attached (*A*) segments were examined and counted separately. Eruption gingivitis was excluded.

Diet investigations.—At the time of the dental examination a questionnaire on types and quantities of foods ingested was completed, in order to estimate the amount of selenium consumed. This was supplemented by a 7-day record of all foods ingested. Any food produced in the area was considered home-produced. To supplement the records of the children, careful inquiries were made at the local food stores. Since Trelease and Beath[7] and Smith and Westfall[11] reported that some foods produced in seleniferous areas are more likely to contain selenium than others, data were obtained on the consumption of the following foods: milk, butter, cheese, eggs, poultry, meat (including game), onions, asparagus, rutabagas, cabbage, peas, beans, tomatoes, beets, cucumbers, potatoes, corn, carrots, wheat, barley, oats, flour, bread, and other baked goods. Utilizing the questionnaire, the 7-day record, and the average of the minimum and maximum selenium content of foods from seleniferous areas,[7, 11] the 24-hour selenium intake was estimated.

Data were obtained on the consumption of foods containing refined sugars such as table sugar, spreads (peanut butter, syrup, honey, jams, and jellies), ice cream and milk drinks, canned and dried fruit, custards and puddings, baked goods, candy, chewing gum, and soft drinks. The frequency of intake and whether these foods were ingested with or between meals were determined.

Analysis.—The quantity of water consumed per day, its source, and the year the supply was first used were recorded. Fluoride and vanadium analyses were made on 145 water samples. Water was not analyzed for selenium because potable water sup-

plies do not contain significant amounts of this element.[7, 37] Fluoride was determined by means of color standards using a slide comparator.* The copper-spark method[41-43] was used for vanadium analyses; the lower level of detectability was about 0.03 ppm.

Twenty-four-hour specimens of urine were obtained from 106 of the 456 children. The urine was collected in half-gallon brown specimen jars containing toluene as a preservative and kept under refrigeration until the time of analysis. The selenium in the urine was determined by the following method of Payne:[44] the urine samples were digested with nitric acid, sulfuric acid, and hydrogen peroxide overnight, then evaporated until charring took place. The liquid then was transferred to a distillation apparatus and treated with hydrobromic acid and an excess of bromine. The resultant distillate was decolorized with sulfur dioxide, transferred to a volumetric flask, and treated with hydroxylamine hydrochloride and gum acacia. The selenium was precipitated in a colloidal elemental form. The final solution was compared in nephelometer tubes with standards prepared the same day in exactly the same manner.

Chi-squares and *t*-tests were used for all tests of hypotheses, to determine statistical significance. All comparisons made in the text with statistical significance are so labeled.

RESULTS AND DISCUSSION

Effect of refined-sugar consumption on variation of dental caries.—There was no consistent variation in the incidence of dental caries of primary and permanent teeth which could be attributed to refined-sugar consumption either with or between meals.

Effect of fluorides in water supplies on incidence of dental caries.—The effect of selenium or vanadium on dental caries susceptibility could not be evaluated without first recognizing the influence of fluoride in the water supplies. The fluoride content of the water supplies ranged from 0.0 to 2.6 ppm. Therefore, it was necessary to divide the groups of children not only into those living in seleniferous and non-seleniferous areas but also according to the concentration of fluoride in the water supplies. The effect of fluoride on primary and permanent teeth is shown in Table 1.

The children who consumed water with a fluoride content ranging from 0.1 to 0.5 ppm showed no reduction in caries incidence in the primary or permanent teeth when compared with those whose water was fluoride-free.

A caries-inhibiting effect of fluoride was noted for those children who consumed water containing from 0.6 to 2.6 ppm fluoride.

A reduction of 33 per cent in the caries attack rates was found in the primary teeth of the children who consumed waters containing 0.6–2.6 ppm of fluoride. This is in accordance with results obtained in endemic fluoride areas by many investigators in this country[45-47] and elsewhere.[48-52]

There was a significant reduction in caries incidence of the permanent teeth for those children whose water supplies contained 0.6 ppm of fluoride and above; 26 per cent for those who lived in seleniferous areas; and 47 per cent for those who lived in non-seleniferous areas. This effect of fluoride is in agreement with a number of investigators[45, 46, 48-57] who have reported significant caries reductions in permanent teeth of children exposed to fluoridated water from birth.

Hereafter, reference will be made to fluoride concentrations of the water as "low fluoride" (0.0–0.5 ppm) and "high fluoride" (0.6–2.6 ppm).

* The W. A. Taylor Company, Baltimore, Md.

TABLE 1

MEAN NUMBER OF DECAYED, MISSING, AND FILLED PERMANENT TEETH (DMFT) AND PRIMARY TEETH (dmft) OF CHILDREN WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES IN NON-SELENIFEROUS AND SELENIFEROUS AREAS

Age	Sex	Fluoride Concentration 0.0–0.5 ppm								Fluoride Concentration 0.6–2.6 ppm							
		Non-seleniferous				Seleniferous				Non-seleniferous				Seleniferous			
		No. Examined	Mean DMFT	No. Examined with Primary Teeth*	Mean dmft	No. Examined	Mean DMFT	No. Examined with Primary Teeth*	Mean dmft	No. Examined	Mean DMFT	No. Examined with Primary Teeth*	Mean dmft	No. Examined	Mean DMFT	No. Examined with Primary Teeth*	Mean dmft
10	Boys	3	6.7	3	5.0	5	4.2	4	6.2	6	1.8	4	2.5	5	3.0	4	7.5
	Girls	5	4.2	4	6.0	10	4.0	9	6.0	4	3.8	4	3.2	9	3.1	7	3.7
11	Boys	6	3.8	6	6.2	9	4.8	5	5.0	7	3.3	5	6.0	6	4.7	5	2.2
	Girls	11	4.9	4	3.0	5	7.0	2	4.0	4	3.8	3	2.7	6	4.5	3	3.3
12	Boys	1	9.0	0	.....	11	8.0	3	4.7	5	2.4	4	4.2	5	3.4	5	5.0
	Girls	7	6.6	1	1.0	7	10.9	1	3.0	4	3.0	2	1.5	5	6.0	2	3.5
13	Boys	6	8.8	0	.....	18	9.4	1	2.0	5	6.4	0	.....	7	6.7	1	2.0
	Girls	6	8.7	1	0.0	10	10.8	0	.....	3	4.7	1	2.0	7	7.9	1	0.7
14	Boys	5	7.0	0	.....	11	10.6	2	1.0	2	0.5	1	3.0	6	8.5	1	4.0
	Girls	5	7.4	0	.....	8	12.4	0	.....	6	3.0	1	0.0	5	10.6	0	.....
15	Boys	7	11.4	0	.....	3	11.0	0	.....	7	4.7	2	2.0	8	11.2	0	.....
	Girls	2	17.5	0	.....	7	16.6	0	.....	4	4.0	1	0.8	8	13.1	0	.....
16	Boys	4	11.5	0	.....	6	13.2	2	6.0	3	11.7	0	.....	2	11.5	0	.....
	Girls	8	10.9	0	.....	6	15.8	1	2.0	6	8.2	1	1.0	3	13.7	0	.....
17	Boys	1	12.0	0	.....	11	13.8	1	2.0	7	6.0	1	0.0	6	8.0	0	.....
	Girls	4	17.0	0	.....	3	26.3	0	.....	2	5.0	0	.....	5	11.6	0	.....
18	Boys	1	17.0	0	.....	3	13.7	0	.....	1	10.0	0	.....	0	.....	0	.....
	Girls	3	12.7	0	.....	0	.....	0	.....	0	.....	0	.....	1	16.0	0	.....
All ages	Boys	34	8.7	9	5.7	77	9.7	18	4.7	43	4.6	17	3.8	45	7.1	16	4.5
	Girls	51	8.6	10	3.6	56	11.6	13	5.2	33	4.5	13	2.0	49	8.4	13	3.4
All ages	Both sexes	85	8.6	19	4.5	133	10.5	31	4.7	76	4.6	30	3.0	94	7.8	29	3.9

* Not exfoliated.

Effect of selenium on incidence of dental caries.—For children who consumed water with a low-fluoride content, there was no appreciable difference in the caries incidence of primary teeth between the non-seleniferous and the seleniferous areas. With a high-fluoride content of the water, there was a mean of 3.0 dmft in the non-seleniferous areas and 3.9 dmft in the seleniferous areas. This would appear to indicate that selenium depressed the beneficial effect of fluoride (Table 1).

Children with permanent teeth who consumed water with a low-fluoride content had a mean of 8.6 DMFT in the non-seleniferous and 10.5 DMFT in the seleniferous areas, a difference of 22 per cent. Those who consumed water with a high-fluoride content had a mean of 4.6 DMFT in the non-seleniferous and 7.8 DMFT in the seleniferous areas, a difference of 70 per cent (Table 1). The higher caries attack rates in seleniferous areas were statistically significant.

In a DMFT unit, one tooth may be both decayed and filled and is counted only once. To provide a more adequate estimate of the severity of the carious attack, not only the carious teeth but also the surfaces with open lesions were considered (Table 2).

TABLE 2

DENTAL CARIES EXPERIENCE OF PERMANENT TEETH FOR 388 CHILDREN WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES IN NON-SELENIFEROUS AND SELENIFEROUS AREAS

	Low Fluoride (0.0–0.5 ppm)		High Fluoride (0.6–2.6 ppm)	
	Non-seleniferous	Seleniferous	Non-seleniferous	Seleniferous
Number of children examined	85	133	76	94
Mean number of DMF teeth	8.6	10.5	4.6	7.8
Mean number of decayed teeth	3.9	7.0	3.5	5.2
Mean number of missing teeth	0.5	0.8	0.03	0.2
Mean number of filled teeth	5.5	4.1	1.5	3.0
Mean number of decayed surfaces	5.4	11.0	4.3	6.9

Children who consumed low-fluoride water had a significantly higher number of decayed teeth and tooth surfaces in the seleniferous than in the non-seleniferous areas. In non-seleniferous areas the mean number of decayed teeth per child was 3.9 and, of decayed surfaces, 5.4. In seleniferous areas, the mean number of decayed teeth was 7.0 and, of decayed surfaces, 11.0.

Children who consumed high-fluoride water and who lived in non-seleniferous areas had a mean number of 3.5 decayed teeth and 4.3 decayed surfaces, compared to 5.2 decayed teeth and 6.9 decayed surfaces in the seleniferous areas. These differences in the number of decayed teeth and surfaces between non-seleniferous and seleniferous areas were statistically significant.

The possible effect of selenium on dental caries was further investigated from the data on selenium intake and selenium excretion. The estimated total daily intake of selenium from food in seleniferous areas ranged from 0.0 to 3.5 mg. None of the children had cereal products originating in the area. Vegetables were rarely home-grown except for onions and potatoes. The main sources of selenium were estimated to be

milk,[7, 11] eggs, poultry,[15] and meat. The selenium content of meat is known to range between 1.7 and 8.0 ppm.[7, 31] The effect of cooking is not known but much of the selenium may be removed by water[7] and discarded.

The relationship between estimated selenium intake and dental caries incidence in this study was investigated (Table 3), but no consistent effect of selenium intake was noted.

Smith[16] found a positive relationship between selenium intake and selenium excretion. The results of this study did not confirm this positive relationship. In fact, at the low level of intake there was evidence of a negative relationship, and the intake was much lower than expected from the data on selenium excretion. However, the determination of urinary selenium is a more objective and accurate means of assessing selenium intake than calculation of the selenium consumed, based on food-intake records.

TABLE 3

MEAN NUMBER OF DECAYED, MISSING, AND FILLED PERMANENT TEETH (DMFT) OF CHILDREN WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES, COMPARED WITH THEIR ESTIMATED DAILY INTAKE OF SELENIUM IN FOOD IN SELENIFEROUS AREAS

Calculated Selenium Intake (mg.)	Low Fluoride (0.0–0.5 ppm)			High Fluoride (0.6–2.6 ppm)		
	No. Children	Per Cent of Children	Mean DMFT	No. Children	Per Cent of Children	Mean DMFT
0.0–0.9.....	57	73.1	10.1	23	46.9	6.3
1.0–3.5.....	21	26.9	8.9	26	53.1	7.4
Total.....	78	.....	9.8	49	.....	6.8

All the urines analyzed contained selenium, and the values were comparable to those reported by Smith, Franke, and Westfall[12] for the seleniferous areas. The highest urinary selenium concentrations found in this study in seleniferous areas were comparable to those reported by Lemley and Merriman[58] for subjects suffering from selenosis.

No urines were collected in non-seleniferous low-fluoride areas. The mean concentration of selenium in the urines of eleven children living in non-seleniferous high-fluoride areas was 0.10 ppm (0.02–0.29 ppm), which is higher than the values reported for non-seleniferous areas by other investigators,[13, 14, 18, 59] but the number of urine samples was too low to give any significance to the relationship between urinary selenium and dental caries incidence.

In the seleniferous areas, concentrations of selenium in the urine ranged from 0.02 to 1.12 ppm, with mean values of 0.05 and 0.16 ppm with low-fluoride and 0.04 and 0.33 ppm with high-fluoride waters (Table 4).

In seleniferous areas with low fluoride, increased concentration of selenium in the urine showed some association with decreased dental caries activity. In seleniferous areas with high fluoride, increased concentration of selenium in the urine showed some association with increased dental caries activity.

The content of selenium in the urine is an indication of selenium intake at, or shortly

preceding, the time when the specimens are collected[16, 59] and not at the time of the onset of carious lesions. Although the children were born and reared in their respective areas and periodic selenium ingestion was highly probable, the pre- and postnatal amounts may have fluctuated considerably. For instance, in one highly seleniferous area where the selenium content of the urines was low and the incidence of dental caries was high, the population had been cautioned to abstain from home-produced food shortly before the present study was undertaken. For this reason, greater weight must be given to the relationship between dental caries incidence and seleniferous areas than between dental caries incidence and the amount of selenium excreted at the time of the urine collection.

Effect of selenium on other oral conditions.—Only two children had enamel hypoplasia of the primary teeth. Both lived in seleniferous areas. Investigations have shown that the percentage of children with enamel hypoplasia of primary teeth is very limited,[60, 61] except in some instances of specialized severe prenatal or neonatal systemic disturbances.[62-64]

TABLE 4

MEAN NUMBER OF DECAYED, MISSING, AND FILLED PERMANENT TEETH (DMFT) OF CHILDREN WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES, COMPARED WITH THEIR URINARY SELENIUM IN SELENIFEROUS AREAS

	Low Fluoride (0.0–0.5 ppm)		High Fluoride (0.6–2.6 ppm)	
	Low Selenium	High Selenium	Low Selenium	High Selenium
Mean selenium concentration (ppm)	0.05 (0.02–0.09)	0.16 (0.10–0.49)	0.04 (0.02–0.08)	0.33 (0.10–1.12)
No. of children examined	63	12	13	5
Mean DFMT	12.1	10.7	7.0	9.6

Enamel hypoplasia of the permanent teeth was detected in an unusually high percentage of children when compared with the investigations of Sarnat and Schour.[60, 61] Of those who consumed water with a low-fluoride content, 22 and 23 per cent were affected in the non-seleniferous and seleniferous areas, respectively; of those who consumed water with a high-fluoride content, 16 and 26 per cent were affected in the non-seleniferous and seleniferous areas, respectively.

Reasons for the high prevalence of hypoplasia among the children of this study are not known. Sarnat and Schour[60, 61] reported that from 5 to 18 per cent of the population is affected with enamel hypoplasia. These investigators and Kreshover, Clough, and Bear[62] have indicated that not all the etiologic factors involved in enamel hypoplasia are known, that any injury of the ameloblasts during enamel formation may cause an arrest in matrix deposition, and that abonormalities of amelogenesis may be considered non-specific in nature.

Chronic endemic dental fluorosis of the permanent teeth was found among 7 per cent of the children examined in non-seleniferous and among 9 per cent in seleniferous areas. None of the primary teeth were affected. Applying Dean's numerical index of weighted severity,[39] the incidence of dental fluorosis was negligible for all areas.

A substantially higher percentage of children had malocclusion in the seleniferous than in the non-seleniferous areas with comparable fluoride content of the water

(Table 5). The highest prevalence of malocclusion (60 per cent) was found among the children living in seleniferous areas whose drinking water had a low-fluoride content. The lowest prevalence (34 per cent) was found in non-seleniferous areas with a high-fluoride content of the water consumed by the children.

No consistent effect of malocclusion on caries incidence of the primary teeth was found in any of the areas (Table 5). However, in the permanent teeth, higher caries attack rates were noted for the children with malocclusion than for those without malocclusion.

A higher percentage of children had gingivitis in the seleniferous than in the non-seleniferous areas (Table 6). Prevalence in non-seleniferous low-fluoride areas—the only areas comparable to other studies—is similar to the results reported by others.[65-68]

Measured by the standards of Massler, Schour, and Chopra,[69] the incidence of

TABLE 5

MEAN NUMBER OF DECAYED, MISSING, AND FILLED PERMANENT TEETH (DMFT) AND PRIMARY TEETH (dmft) OF CHILDREN WITH AND WITHOUT MALOCCLUSION WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES IN NON-SELENIFEROUS AND SELENIFEROUS AREAS

	Low Fluoride (0.0–0.5 ppm)				High Fluoride (0.6–2.6 ppm)			
	Non-seleniferous		Seleniferous		Non-seleniferous		Seleniferous	
	Without Malocclusion	With Malocclusion	Without Malocclusion	With Malocclusion	Without Malocclusion	With Malocclusion	Without Malocclusion	With Malocclusion
Number of children	50	35	53	80	50	26	56	38
Mean DMFT	7.9	9.6	9.2	11.6	4.5	4.5	7.2	8.6
Number of children with primary teeth*	12	7	13	18	18	12	19	10
Mean dmft	4.8	4.6	5.5	3.4	3.3	2.8	3.5	4.9

* Not exfoliated.

TABLE 6

MEAN NUMBER OF DECAYED, MISSING, AND FILLED PERMANENT TEETH (DMFT) AND PRIMARY TEETH (dmft) OF CHILDREN WITH AND WITHOUT GINGIVITIS WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES IN NON-SELENIFEROUS AND SELENIFEROUS AREAS

	Low Fluoride (0.0–0.5 ppm)				High Fluoride (0.6–2.6 ppm)			
	Non-seleniferous		Seleniferous		Non-seleniferous		Seleniferous	
	Without Gingivitis	With Gingivitis	Without Gingivitis	With Gingivitis	Without Gingivitis	With Gingivitis	Without Gingivitis	With Gingivitis
Number of children	52	33	38	95	37	39	44	50
Mean DMFT	7.6	9.4	7.6	11.0	3.6	5.3	7.7	7.6
Number of children with primary teeth*	11	8	13	18	20	10	10	19
Mean dmft	4.9	4.4	3.7	5.6	3.2	2.9	4.3	3.8

* Not exfoliated.

gingivitis (the PMA index) was severe in all areas and most severe in the seleniferous low-fluoride areas (Table 7). It was much higher than the incidence reported for similar ages by other investigators who used the PMA index and included eruption gingivitis.[65, 69, 70] Comparable to the investigations by Schour and Massler,[40] Massler *et al.*,[69] and Savara,[65] papillary units were those affected most frequently, and the number of affected attached units was negligible. The maxillary gingiva was more frequently affected than the mandibular gingiva. The difference was statistically significant.

TABLE 7

INCIDENCE OF GINGIVITIS FOR 388 CHILDREN WITH 0.0–0.5 AND 0.6–2.6 PPM OF FLUORIDE IN THEIR WATER SUPPLIES IN NON-SELENIFEROUS AND SELENIFEROUS AREAS

	Low Fluoride (0.0–0.5 ppm)		High Fluoride (0.6–2.6 ppm)	
	Non-seleniferous	Seleniferous	Non-seleniferous	Seleniferous
Mean no. papillary units affected:				
Maxillary units	3.3	3.8	3.7	3.2
Mandibular units	2.9	3.7	2.4	2.9
Mean no. marginal units affected:				
Maxillary units	2.5	2.3	1.7	1.2
Mandibular units	0.6	1.2	0.8	0.9
Mean no. attached units affected:				
Maxillary units	0.3	0.3	0.7	0.6
Mandibular units	0.3	0.1	0.2	0.1
Mean no. P+M+A units affected:				
Maxillary units	6.1	6.4	6.1	5.0
Mandibular units	3.9	5.0	3.4	3.9
Maxillary and mandibular units	10.1	11.4	9.5	8.9

Decreases of vitamin A and ascorbic acid in blood and liver of livestock due to the ingestion of selenium have been reported.[71, 72] None of the oral hyperkeratotic lesions which may accompany avitaminosis A, if it is of long standing,[73] were found among the children of this study. In a seleniferous area, only one case was seen which appeared to have the hemorrhagic, hyperplastic gingivitis and mobile teeth typical of frank ascorbic acid deficiency[73] coupled with a poor diet and poor oral hygiene.[70, 74, 75] Kreshover,[74] Glickman,[75] Zappler,[68] and Parfitt[70] found that an increased demand for a nutrient, if not met, may affect the metabolism of periodontal tissues, especially when combined with local irritants. Because of a possible increased demand in seleniferous areas, subclinical deficiencies of these vitamins may have been predisposing factors in the increased prevalence of gingivitis among the children living in seleniferous areas.

No consistent relationship was found between gingivitis and dental caries of the primary teeth or for permanent teeth in the seleniferous high-fluoride areas. However, in all other areas dental caries activity of the permanent teeth was significantly higher for the children with gingivitis than for those without gingivitis (Table 6). Like the prevalence of gingivitis, the incidence of dental caries of the children with gingivitis was significantly increased by selenium.

Effect of vanadium on incidence of dental caries.—Vanadium was found in all specimens of the water supplies consumed by the children. Only minute amounts were discovered, ranging from 0.03 to 0.22 ppm, with the majority between 0.03 and 0.09 ppm. These concentrations are much lower than those used experimentally by Geyer,[23] Hein and Wisotzky,[24] and Muhler.[26]

In the non-seleniferous areas, only the lower ranges between 0.03 and 0.09 ppm of vanadium were found. In the seleniferous areas, a higher vanadium content was discovered in some of the water supplies. This substantiates the investigations of Trelease and Beath,[7] who reported vanadium deposits in connection with the selenium ores.

In this study the children were classified into the following divisions:

Non-seleniferous

1. Low fluoride (0.0–0.5 ppm)
   a) Vanadium, 0.03–0.06 ppm
   b) Vanadium, 0.07–0.09 ppm
2. High fluoride (0.6–2.6 ppm)
   a) Vanadium, 0.03–0.06 ppm
   b) Vanadium, 0.07–0.09 ppm

Seleniferous

1. Low fluoride (0.0–0.5 ppm)
   a) Vanadium, 0.03–0.06 ppm
   b) Vanadium, 0.07–0.09 ppm
   c) Vanadium, 0.10–0.22 ppm
2. High fluoride (0.6–2.6 ppm)
   a) Vanadium, 0.03–0.06 ppm
   b) Vanadium, 0.07–0.09 ppm
   c) Vanadium, 0.10–0.22 ppm

In all areas, a decrease in caries activity of the primary dentition was found with an increase of vanadium concentration in the water (Table 8). However, because of the small number of children with primary teeth in each of the groups who consumed water containing vanadium, the reduction in dental caries rates was not significant.

In non-seleniferous areas there seemed to be an inverse relationship between caries activity of the permanent dentition and vanadium concentration. However, here also, the number of subjects was not sufficient to draw any conclusions as to the effect of vanadium on the caries rates of children (Table 8).

In seleniferous areas the dental caries rates of the children who consumed water with 0.07–0.09 ppm of vanadium were considerably less than the caries rates of the children whose water contained vanadium at the lowest level of detectability (0.03–0.06 ppm). Reductions of dental caries by vanadium were 17 per cent with low and 19 per cent with high fluoride. Caries activity was still further decreased by vanadium at its highest concentrations (0.10–0.22 ppm) and low fluoride in the water. However, with the highest concentration of vanadium and high fluoride in the water, dental caries rates were increased; but, when the dental caries activity of these children was compared to the caries rates of the children who consumed water with vanadium at its lowest concentration, a slight reduction was observed.

Even though statistical significance could not be demonstrated in the four areas individually, the reduction in dental caries rates by vanadium was significant over the combined areas. It can be concluded, therefore, that vanadium, even in concentrations as low as 0.07–0.09 ppm, reduced the dental caries rates.

SUMMARY AND CONCLUSIONS

The effect of selenium and vanadium on the dental caries incidence of children ten to eighteen years of age in selected seleniferous and non-seleniferous areas was

TABLE 8

MEAN NUMBER OF DECAYED, MISSING, AND FILLED PERMANENT TEETH (DMFT) AND PRIMARY TEETH (dmft) OF CHILDREN, 10 TO 18 YEARS OF AGE, COMPARED WITH VARIOUS CONCENTRATIONS OF VANADIUM WITH FLUORIDE CONTENTS OF 0.0–0.5 AND 0.6–2.6 PPM IN THEIR WATER SUPPLIES IN NON-SELENIFEROUS AND SELENIFEROUS AREAS

Vanadium Concentration (ppm)	Non-seleniferous								Seleniferous							
	Low Fluoride (0.0–0.5 ppm)				High Fluoride (0.6–2.6 ppm)				Low Fluoride (0.0–0.5 ppm)				High Fluoride (0.6–2.6 ppm)			
	No. Children Examined	Mean DMFT	No. Children Examined with Primary Teeth*	Mean dmft	No. Children Examined	Mean DMFT	No. Children Examined with Primary Teeth*	Mean dmft	No. Children Examined	Mean DMFT	No. Children Examined with Primary Teeth*	Mean dmft	No. Children Examined	Mean DMFT	No. Children Examined with Primary Teeth*	Mean dmft
0.03–0.06	76	8.9	15	5.1	75	4.6	29	3.1	44	11.9	11	5.0	10	8.8	3	7.0
0.07–0.09	9	6.4	4	3.2	1	2.0	1	1.0	79	9.9	18	5.0	48	7.1	18	3.8
0.10–0.22	0	.....	0	.....	0	.....	0	.....	10	8.9	2	1.0	36	8.4	8	3.2

* Not exfoliated.

investigated. The effect of selenium on hypoplasia, malocclusion, and gingivitis was also studied.

The individual water supplies were found to contain fluoride concentrations ranging from 0.0 to 2.6 ppm. No reduction in dental caries rates of the primary and permanent teeth was found with fluoride concentrations from 0.1 to 0.5 ppm, but reductions were observed in all areas with fluoride concentrations from 0.6 to 2.6 ppm.

Dental caries rates of the permanent teeth were significantly higher in seleniferous areas than in non-seleniferous areas.

The estimated selenium intake from foods was found to be well within the safety limits. Although all urines analyzed contained selenium, no consistent relationship was found between the intake and excretion of selenium, between intake and dental caries, or between excretion and dental caries.

No consistent variation in the incidence of dental caries was found which could be attributed to sugar consumption.

Two children in seleniferous areas had enamel hypoplasia of the primary teeth. An unusually large percentage of children had enamel hypoplasia of the permanent teeth, with the largest percentage in seleniferous areas with a high-fluoride content of the water.

The incidence of dental fluorosis was negligible among the children.

Prevalence of malocclusion was increased in seleniferous areas and decreased with high concentrations of fluoride in the water. No consistent relationship existed between malocclusion and caries incidence of the primary teeth. However, a positive relationship was found between malocclusion and caries incidence of the permanent teeth.

A higher percentage of children had gingivitis in the seleniferous than in the non-seleniferous areas. The gingivitis was severe, and the highest number of PMA units affected were found in the seleniferous low-fluoride areas. No consistent relationship was found between gingivitis and caries incidence of the primary teeth, but a positive relationship existed between gingivitis and caries incidence of permanent teeth.

Vanadium was found in all water samples analyzed, ranging in concentrations from 0.03 to 0.22 ppm. No vanadium concentrations above 0.09 ppm were detected in non-seleniferous areas. In the primary teeth there appeared to be a trend toward decreasing caries activity with increasing vanadium concentrations. In the permanent teeth, there was a decrease in dental caries rates with increasing vanadium concentrations which was significant for all areas combined.

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Nutrition and Caries
XII. *Endogenic versus Exogenic Factors*

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Local-acting (exogenic) factors are known to be very important in producing caries. Their importance has recently been emphasized in studies concerned with the role of the oral flora, including experiments using germ-free animals,[1] antibiotics,[2-4] EDTA,[5] and work testing the proteolysis-chelation theory.[6] Experiments in which the local cariogenic action of food was reduced by tube-feeding,[7, 8] by giving fluid or semifluid food,[9-13] or by substituting semifluid dairy products for part of a cariogenic, highly purified ration[14] further illustrate the importance of exogenic factors. Studies concerned with the effects of food restriction,[10, 15, 16] the effects of reducing the size of the particles in the rations,[17, 18] extraction of part of the teeth,[19-21] and the effects of desalivation[16, 22-24] can also be mentioned in this regard. Investigations on parabionts* also illustrate the importance of local factors.[25, 26] Studies in which the frequency of meals was varied revealed a reduction in caries when the number of meals was reduced.[27, 28]

The importance of endogenic factors can be seen from studies dealing with the influence of heredity.[29] Prenatal and postnatal factors which influence tooth formation and maturation will also determine the resistance of the erupted tooth to harmful influences.

In general, it is clear that both endogenic and exogenic factors play a role, but which of these are more important in causing dental caries is less clear. Specifically, this study was concerned with the possibility of preserving, by dietary means, noncarious teeth in a caries-free state, in spite of the presence of caries-susceptible dental tissue.

MATERIALS AND METHODS

The compositions of the two experimental diets are given in Table 1. The RAN (cariogenic) diet was composed according to the real average consumption of the Dutch population; all ingredients were, however, dried and ground. Studies of nutrition and caries using this diet are numerous.[12, 15, 30-32] The PATC (non-cariogenic) diet was a variant of the PA diet used by Bonting.[33] Like the PA diet, it had a very low cariogenicity, but it had a higher content of whole wheat and minerals than did the original PA diet. The mineral supply added to the PATC diet was a salt mixture

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* Two animals (rats) united together by the scapulae, ventral muscle, and peritoneum. A daily blood interchange of at least ten times the blood volume is established after 3 or 4 days of parabiotic union.

described by Constant, Sievert, Phillips, and Elvehjem.[34] These authors reported that these salt supplements had a caries-reducing effect.

Experiment 1.—Five first-generation rats (2 males, 3 females) were fed the non-cariogenic (PATC) diet, and 15 first-generation rats (10 males, 5 females) were fed the highly cariogenic (RAN) diet from the age of weaning. After reaching maturity, the animals were mated with members of their own group, and further investigations were carried out with the offspring.

Twenty offspring of the first-generation animals fed the PATC diet were divided into two groups of 10 animals each (7 males, 3 females). One group of offspring (group PATC-PATC) received the *same* diet that its parents had received, and the other group (group PATC-RAN) was fed the diet that its parents had *not* received. Likewise, 24 offspring of the first-generation animals fed the RAN diet were divided into two groups (RAN-RAN and RAN-PATC) of 12 animals each (7 males, 5 females)

TABLE 1

COMPOSITION OF DIETS

Composition of RAN diet (per cent):	
Bread (dried)	41.4
Rolled oats	0.45
Groats (barley)	0.55
Corn flour	0.65
Rice	1.5
Potatoes (dried)	11.35
Potato flour	0.75
Sugar	16.2
Glucose	1.4
Milk powder (whole)	9.0
Skim-milk powder	1.4
Meat (dried)	2.4
Fish (dried)	1.1
Cheese	2.2
Eggs	1.6
Pulses	0.9
Vegetables (dried)	1.85
Apples (dried)	2.55
Hazel nuts	0.1
Peanuts	0.15
Cocoa	1.1
Salt (NaCl)	1.4
Total	100
To this base diet is added 15.5% of a mixture (in per cent) composed of:	
Margarine	52.7
Butter	8.7
Salad oil	5.2
Beef fat	17.4
Lard	16

Composition of PATC diet (per cent):	
Casein	6
Dried full milk	10
Whole-wheat flour	66
Peanut oil	6.5
Cod-liver oil	0.5
Brewer's yeast	5
Salt mixture	4
Extra salts (C-salts[34])	2
Total	100
Salt mixture (gm.):	
$Ca_3(PO_4)_2$	20.0
$Na_2HPO_4 \cdot 2aq$	8.0
KCl	10.0
$MgCO_3$	2.0
Na-citrate	1.0
Various salts (trace elements)	1.0
Trace-element mixture (gm.):	
Sodium silicate	1,000
Ferrous sulfate	650
Potassium-aluminum sulfate	420
Zinc sulfate	250
Copper sulfate	60
Manganese sulfate	60
Nickel sulfate	20
Cobalt sulfate	10
Potassium bromide	50
Sodium fluoride	20
Sodium molybdate	10
Borax	10
Potassium iodide	1
Arsenic	0.5
C-salts (gm.):	
CaO	154
K_2HPO_4	185.6
KCl	61.9
MgO	13.3
$Na_2PO_4 \cdot 12aq$	290.5

and fed their respective diets according to the above-mentioned procedure. The experimental dietary measures were initiated when the offspring were 1 month old.

The month after this procedure, increases in weight were recorded, and caries was scored for all four groups of offspring.

Experiment II.—As in the first experiment, the offspring of first-generation animals fed either the PATC or the RAN diet were divided into four groups when 1 month old; two groups (RAN-RAN, 7 males and 6 females; PATC-PATC, 5 males and 6 females) received the same diet as their parents had received, and two groups (PATC-RAN, 5 males and 4 females; RAN-PATC, 6 males and 6 females) were fed the diet that their parents had not received. To all four groups the diet was given for another 2 months. Two more groups—RAN-RAN (5 males, 2 females) and RAN-PATC (6 males, 9 females)—were studied after a 4.5-month period. Weight increases were recorded after 1 and 2 months and at the end of 4.5 months for groups RAN-RAN and RAN-PATC. Caries was recorded 2 and 4.5 months after dividing into four dietary groups.

The parent animals were evaluated for caries after 10 months on the experimental diets, and their weight gains after 1, 2, and 4.5 months were observed.

Caries was scored using the microscopic evaluation method described previously (Fig. 1).[35] A modification was used of the apparatus described by Jansen,[36] consisting of a circular saw blade of phosphobronze, 0.04 mm. thick, provided with a large central opening. The saw blade is mounted in a drum, which is rotated at about 6,000 rpm. The inner rim of the blade is the cutting edge. Diamond powder is applied to make the cutting of non-decalcified dental tissue possible (Figs. 2 and 3).

After dissection of the jaws, fixation in 10 per cent formalin, and inspection in order to record the gross lesions (binocular loupe 15×), the bone material and incisors were removed as far as possible, leaving the three molars with the adjacent bone tissue only. The thus prepared jaw quadrants were mounted on the moveable object-holder of the machine with "stents" (plastic material which softens on warming). A cut was made in longitudinal directions through all three molars, so as to obtain two halves of each jaw quadrant. This cutting procedure takes only seconds.

Carious processes can be stained with many dyes. Aqueous fuchsin 1 per cent was used routinely. The extent of the lesions was measured by means of an ocular micrometer inserted into a microscope (mag. ± 60). In the cut surface the extension of the lesion in the mesiodistal and occlusiogingival direction was measured (in ocular-micrometer units). The bucco-lingual extension was estimated by the presence of the lesion in one or both of the jaw quadrant halves. (More accurate estimations can be obtained by cutting more sections.) Multiplying the figures obtained for these dimensions gave a measure for the volumes of the lesions. A distinction was made between lesions with pathological staining only (initial lesions) and lesions with loss of surface continuity (cavities). Caries in the enamel, dentino-enamel junction, and dentin was measured and recorded separately, and for fissure lesions as well as for approximal lesions.

Statistical calculations on growth gains and caries data were carried out with Wilcoxon's[37-39] test and the test of Kruskal and Wallis.[40]

RESULTS

Growth.—In general, the results indicated better growth in animals receiving the PATC diet (Table 2). Furthermore, groups PATC-RAN showed significantly greater gains in weight than did the RAN-RAN groups.

Caries.—In Experiment I (Table 3) the differences in fissure caries between the groups were highly significant. The animals on the RAN diet had significantly more carious tissue than had the animals on the PATC diet (bilateral tail error $P_2 \ll 0.001$ for both enamel and dentino-enamel junction attack).

Group RAN-RAN had significantly more caries than did group PATC-RAN ($P_2 < 0.01$ for the dentino-enamel junction scores; $P_2 \ll 0.05$ for the enamel scores). No significant difference was found, however, between groups RAN-PATC and PATC-PATC. This might have been due, in part, to the small number of lesions and low caries scores obtained from these groups.

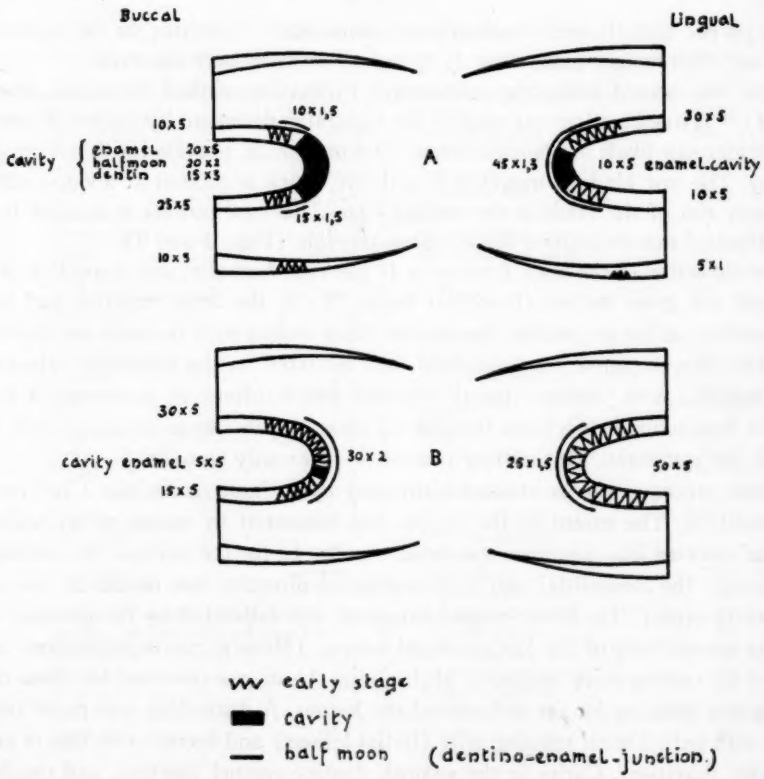

FIG. 1.—Example of the recording of lesions and the calculation of scores. *Score of lesion A:* enamel—attacked $55 \times 5 \times 2$, from this is cavity $20 \times 5 \times 2$; half-moon—attacked $45 \times 1.7 \times 2$, from this is cavity $20 \times 2 \times 1$; other dentin—$15 \times 3 \times 1$. *Score of lesion B:* enamel—attacked $50 \times 5 \times 2$, from this is cavity $5 \times 5 \times 1$; half-moon—attacked $30 \times 2 \times 2$. *Score of approximal lesion:* $10 \times 5 \times 2$. (For the sake of convenience the half-moon is not sketched in *on* the dentino-enamel junction, but somewhat lower in the dentin.) (Reprinted from L. M. Dalderup, Evaluation of Dental Caries in the Rat in Non-decalcified Sections of the Molars, *Intern. Rev. Vitamin Research*, **26**:251, 1955.)

Involvement of the dentin was present in 5 of the RAN-RAN animals and in only 1 of the PATC-RAN animals. Most of these lesions were found in the mandibula.

In the above-mentioned statistical calculations, only the *extent* of the lesions was taken into account; the severity was not considered. In 6 animals of the RAN-RAN groups and in 1 animal of the PATC-RAN group, cavities were observed. Most of these cavities were seen in the mandibula, as is usually the case when caries is quite extensive.

In both RAN-fed groups, the mandibula had significantly more dental caries of the

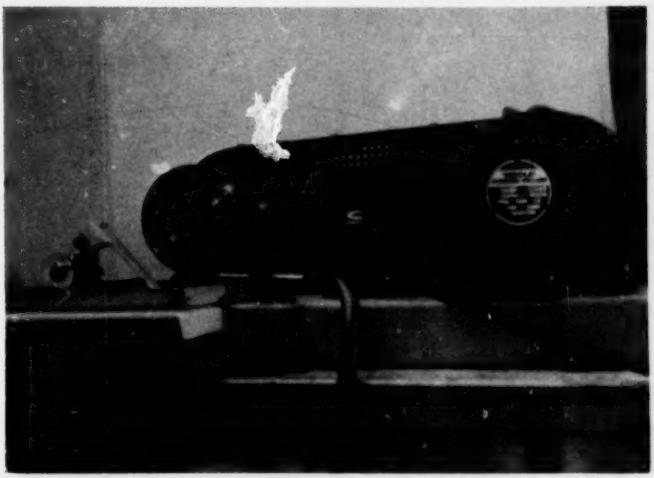

FIG. 2.—Cutting machine used for the preparation of non-decalcified sections of the jaw quadrants. The drum, containing the circular saw blade, is covered with a protective cap which prevents the cooling water (*coming from the right side*) from splashing about. The movable object-holder is attached on the left side. Object-holder and cooling equipment are in working position.

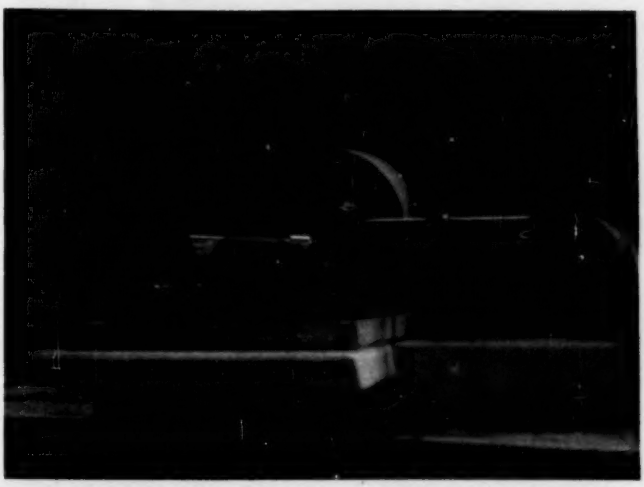

FIG. 3.—Cutting machine with cap removed and drum and saw blade exposed

TABLE 2

WEIGHT INCREASES IN GRAMS

	RAN-RAN		PATC-RAN		RAN-PATC		PATC-PATC	
	Male	Female	Male	Female	Male	Female	Male	Female
Experiment I animals:								
After 1 month	93	80	111	92	130	97	142	103
Experiment II animals:								
After 1 month	97	87	137	94	130	90	126	92
After 2 months	206	131	236	136	243	139	237	147
After 4.5 months	286	157	.....	.....	298	196	.....	.....
	RAN		PATC					
Parent animals:								
After 1 month	77	71	138	78				
After 2 months	157	115	257	100				
After 4.5 months	256	160	360	180				

TABLE 3

EXPERIMENT I: CARIES EVALUATION AFTER 1 MONTH

	AVERAGE CARIES SCORE			
	RAN-RAN	PATC-RAN	RAN-PATC	PATC-PATC
MAXILLA				
Fissure attack:				
Enamel	1,880 (7.5)*	1,000 (6.8)	356 (4.6)	385 (4.0)
Dentino-enamel junction	133.9 (6.9)	12.4 (2.1)	0.5 (0.3)	.....
Dentin	5 (0.1)	.....	.....	.....
Fissure cavity:				
Enamel	109 (0.3)	.....	.....	.....
Dentino-enamel junction	7.3 (0.2)	.....	.....	.....
Dentin	5 (0.1)	.....	.....	.....
Approximal attack:				
Enamel	44 (1.4)	13 (1.0)	16 (0.7)	16 (0.7)
Dentino-enamel junction	0.4 (0.3)	0.1 (0.1)	.....	.....
Dentin	.....	16 (0.1)	.....	.....
Approximal cavity:				
Enamel	0.5 (0.1)	.....	.....	.....
Dentino-enamel junction	.....	.....	.....	.....
Dentin	.....	.....	.....	.....
MANDIBULA				
Fissure attack:				
Enamel	2,307 (9.8)	1,354 (7.6)	472 (4.8)	329 (3.7)
Dentino-enamel junction	286.0 (8.1)	58.7 (4.3)	0.3 (0.4)	.....
Dentin	45 (0.5)	5 (0.1)	.....	.....
Fissure cavity:				
Enamel	220 (1.0)	15 (0.2)	.....	.....
Dentino-enamel junction	28.4 (0.7)	2.4 (0.2)	.....	.....
Dentin	45 (0.5)	5 (0.1)	.....	.....
Approximal attack:				
Enamel	67 (2.1)	57 (1.3)	34 (1.9)	37 (1.3)
Dentino-enamel junction	0.1 (0.2)	.....	.....	.....
Dentin	.....	.....	.....	.....
Approximal cavity:				
Enamel	.....	6 (0.1)	.....	.....
Dentino-enamel junction	.....	.....	.....	.....
Dentin	.....	.....	.....	.....

* Figures in parentheses indicate average number of lesions.

enamel and dentino-enamel junction than the maxilla ($P_2 \ll 0.01$). Caries of the dentin was not frequent enough to allow statistical calculations to be made. In the PATC-fed animals, no significant difference was found between the caries scores of the maxilla and mandibula.

Approximal lesions were much less frequent and of slight severity. The differences between the groups were small and not statistically significant, although the average value for the RAN-RAN groups was somewhat higher. Almost all lesions occurred in the enamel and appeared chalky white and less translucent than normal tissue; the cutting surface, in some instances, appeared rougher and softer than sound enamel. Significantly less caries was present in the right upper quadrant than in the other jaw quadrants of the RAN-fed groups ($P_2 < 0.05$). In none of the other groups was such a difference found.

In Experiment II the results concerning the amount of fissure caries after an experimental period of 2 months were almost the same as in the first experiment after a 1-month period (Table 4). The animals on the RAN diet had significantly more

TABLE 4

EXPERIMENT II: CARIES EVALUATION AFTER 2 MONTHS

	AVERAGE CARIES SCORE			
	RAN-RAN	PATC-RAN	RAN-PATC	PATC-PATC
MAXILLA				
Fissure attack:				
Enamel	1,201 (6.6)*	989 (6.3)	618 (5.0)	411 (3.0)
Dentino-enamel junction	77.8 (4.1)	3.4 (1.9)	3.6 (1.7)	2.5 (1.2)
Dentin	33 (0.1)	.....	.....	.....
Fissure cavity:				
Enamel	264 (0.6)	.....	.....	.....
Dentino-enamel junction	66.8 (0.6)	.....	.....	.....
Dentin	33 (0.1)	.....	.....	.....
Approximal attack:				
Enamel	130 (1.5)	21 (0.7)	20 (0.6)	5 (0.2)
Dentino-enamel junction	0.7 (0.1)	.....	.....	.....
Dentin	2 (0.1)	.....	.....	.....
Approximal cavity:				
Enamel	5.6 (0.1)	.....	.....	.....
Dentino-enamel junction	0.7 (0.1)	.....	.....	.....
Dentin	2 (0.1)	.....	.....	.....
MANDIBULA				
Fissure attack:				
Enamel	2,339 (8.9)	1,375 (8.6)	660 (4.4)	334 (3.1)
Dentino-enamel junction	527.0 (6.5)	29.8 (4.6)	7.5 (2.4)	1.2 (0.6)
Dentin	593 (1.4)	.....	.....	.....
Fissure cavity:				
Enamel	1,092 (2.3)	.....	.....	.....
Dentino-enamel junction	452.6 (2.0)	.....	.....	.....
Dentin	593 (1.4)	.....	.....	.....
Approximal attack:				
Enamel	30 (1.1)	19 (0.7)	5 (0.4)	4 (0.5)
Dentino-enamel junction	.....	.....	.....	.....
Dentin	.....	.....	.....	.....
Approximal cavity:				
Enamel	.....	.....	.....	.....
Dentino-enamel junction	.....	.....	.....	.....
Dentin	.....	.....	.....	.....

*Figures in parentheses indicate average number of lesions.

caries than the PATC-fed animals ($P_2 \ll 0.001$ for both enamel and dentino-enamel junction lesions). Differences in extent of carious enamel between groups from the same parental stock were not significant. The difference in extent of caries in the dentino-enamel junction, however, was significant between groups RAN-RAN and PATC-RAN ($P_2 \ll 0.05$) and approached significance between the other two groups.

Carious dentin was present in only 6 of the 12 animals of group RAN-RAN.

Cavities were found only in group RAN-RAN, indicating a highly significant difference between this group and group PATC-RAN when only the *severity* of the lesions was considered. The extent of the lesions was, of course, limited, as the amount of available enamel tissue was limited.

Using severity alone as the index, a significant difference was found for the enamel lesions and dentino-enamel junction attack between the RAN-fed and PATC-fed groups.

Again, caries attack in the mandibula was significantly greater than in the maxilla in the RAN-fed groups ($P_2 \ll 0.001$), but not in the PATC-fed groups.

As in the first experiment, approximal lesions were less severe than the fissure lesions. The differences between the RAN-fed and PATC-fed groups were statistically significant ($P < 0.05$). The RAN-diet groups, group RAN-RAN especially, had more extensive caries than the PATC-diet groups. No single jaw quadrant showed more extensive caries than the other quadrants.

After 4.5 months (Table 5), the difference in number of fissure enamel and dentino-enamel junction lesions between groups RAN-RAN and RAN-PATC was highly significant ($P_2 \ll 0.001$). Moreover, all RAN-RAN animals had one or more cavities; the others had none. Carious dentin was present only in the RAN-RAN animals. Caries in the mandibula was significantly more severe than in the maxilla in group RAN-RAN ($P_2 \ll 0.05$), but not in group RAN-PATC. The difference in number of approximal lesions was not significant between the two groups.

Comparison of data obtained after 1, 2, and 4.5 months.—In the RAN-RAN groups no significant increase in the extent of fissure enamel lesions with time was evident. Extent of enamel attack is limited because the amount of enamel is limited. Attack was early and extensive in all animals on the RAN ration. There was, however, a significant increase in severity when judged by the number of cavities ($P_2 \ll 0.01$).

An increasingly extensive ($P_2 \ll 0.01$) and severe ($P_2 \ll 0.001$, when number of cavities was counted) carious attack of the dentino-enamel junction with time was observed.

Too few lesions were present in the dentin to make calculations possible.

No significant increase in number of extent of approximal lesions with time could be seen.

In the PATC-RAN groups no significant increase was observed in the extent of carious attack in the fissures or approximal surfaces after 2 months.

In the RAN-PATC groups no significant increase after 4.5 months was seen in the extent of enamel fissure attack; however, scores obtained after 2 months were higher than scores obtained after 1 month. Caries scores of the dentino-enamel junction were highest after 2 months ($P < 0.05$). No carious dentin and cavities were observed.

Differences in scores of approximal enamel were not significant. No lesions were found at other sites.

In the PATC-PATC groups no significant differences in fissure and approximal enamel attack after 2 months were demonstrated. No lesions were found at other sites.

The number of fissure enamel lesions was significantly higher in the RAN-fed groups after 1, 2 ($P_2 \ll 0.001$), and 4.5 months ($P_2 \ll 0.01$). The increase in number of lesions with time in the RAN-RAN animals was not significant. In none of the other three diet groups were significant increases seen in the number of enamel lesions. (P was not calculated for lesions at the other sites because they were too few in number.)

The two groups of parent animals had, as could be expected, a highly significant difference in caries scores. In fact, the teeth of the RAN-fed parent animals were so

TABLE 5

EXPERIMENT II: CARIES EVALUATION AFTER 4.5 MONTHS

	AVERAGE CARIES SCORE	
	RAN-RAN	RAN-PATC
MAXILLA		
Fissure attack:		
Enamel	1,496 (8.0)*	512 (5.6)
Dentino-enamel junction	302.1 (6.4)	0.7 (0.5)
Dentin	.....	.....
Fissure cavity:		
Enamel	504 (1.3)	.....
Dentino-enamel junction	221.2 (1.1)	.....
Dentin	.....	.....
Approximal attack:		
Enamel	11 (0.5)	16 (0.9)
Dentino-enamel junction	.....	.....
Dentin	.....	.....
Approximal cavity:		
Enamel	.....	.....
Dentino-enamel junction	.....	.....
Dentin	.....	.....
MANDIBULA		
Fissure attack:		
Enamel	2,406 (9.6)	477 (5.2)
Dentino-enamel junction	836.6 (7.0)	8.6 (1.3)
Dentin	265 (0.7)	.....
Fissure cavity:		
Enamel	1,577 (3.7)	.....
Dentino-enamel junction	716.5 (3.3)	.....
Dentin	265 (0.7)	.....
Approximal attack:		
Enamel	55 (1.0)	20 (1.1)
Dentino-enamel junction	.....	.....
Dentin	.....	.....
Approximal cavity:		
Enamel	.....	.....
Dentino-enamel junction	.....	.....
Dentin	.....	.....

* Figures in parentheses indicate average number of lesions.

destroyed by caries that microscopic evaluation was not possible. The PATC-fed parent animals yielded very low caries scores, and no cavities were found in this group.

Differences in caries scores and numbers of lesions between sexes were not found.

DISCUSSION

In concordance with our other experiments, it was found that the number and extent of lesions are determined very early in the experimental period. Beyond 1 month on the cariogenic diet, an increase in severity is the most outstanding feature and results in cavity formation if the diet is highly cariogenic.

The increasing severity of caries during the 4.5-month experimental period was much more evident in the mandibula than in the maxilla. The number of lesions in the maxilla and the mandibula, however, did not differ greatly.

Of practical significance is the finding that initially caries-immune teeth can offer partial resistance against the influence of a cariogenic diet, as evidenced by the finding that offspring from mothers on the non-cariogenic PATC diet developed less caries on the cariogenic RAN diet than did the offspring of RAN-fed parents. On the other hand, the beneficial effects of the non-cariogenic PATC diet given to offspring at the age of 1 month were so great that no significant differences were found between offspring of the RAN-fed parents and those of the PATC-fed parents. There were, however, indications of a somewhat more favorable condition of the molars in the latter group.

Some lesions (spots with slight affinity to stain with fuchsin), however, were found, perhaps indicating an inadequately advanced maturity of the teeth,[7] for enamel deep in the fissures matures later than that in the cusps. Fissure cavities have never been observed in animals on the PATC ration or in those consuming the PA diet.

It should be remarked that the diets used in this experiment, although based on data of human consumption, differed from the actual human diet in consistency and methods of preparation. In the usual dried and ground form, the RAN diet is probably more cariogenic than is the fresh form used for human consumption, since cooking with water decreases its cariogenicity.[12] The coarseness of the particles in both diets in the form used in these experiments did not show important variations. A special factor in the PATC diet which may have an anticariogenic action has not yet been isolated in experiments with rats. Undoubtedly, the mineral and vitamin content of the PATC diet was higher than that of the RAN diet. Omitting the salts or yeast powder from the original PA diet, however, did not appear to affect the cariogenicity of the PATC diet.

The results shown here stress once more the relatively enormous effect of local influences, either harmful or beneficial, on the teeth.

Growth differences between the various groups closely paralleled differences in caries scores—better growth correlated with better teeth. Such a relation, however, has not been a constant finding in caries research, as is demonstrated by the effects of food restriction.[10, 15, 16]

In general, growth on RAN food is less than on more natural diets such as PATC, and food intake is higher on the latter diets. So a small food intake cannot play a role in the low cariogenicity of the PATC food. Nor does the higher food intake and weight gain of male animals make them more caries-susceptible than females.

SUMMARY

Caries was evaluated in two generations of rats fed either a cariogenic (RAN) or non-cariogenic (PATC) diet in an attempt to assay the relative importance of endogenic and exogenic factors in the carious process.

The animals fed the non-cariogenic diet from their fourth week of life had significantly less caries than those fed the cariogenic ration. Offspring from parents fed the non-cariogenic diet proved to be somewhat protected against the cariogenic effects of the RAN diet; however, offspring fed the non-cariogenic diet, although their parents had been fed the cariogenic ration, showed much greater resistance to caries than did offspring fed the cariogenic diet whose parents had received the non-cariogenic diet. Growth was significantly greater in animals consuming the non-cariogenic ration.

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Distribution of Dental Caries in Children

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An attempt to obtain the statistical distributions of dental caries has been made by Grainger and Reid.[1]* Their method consists of fitting the well-known distributions such as the negative binomial to sample data and selecting the ones which give close fits. The principal disadvantage in using these distributions is that the assumptions underlying their formulation are hardly satisfied in any practical situation, and hence, even with a good fit, their utility in analyzing data is highly restricted. An alternative to this method is to formulate distributions on the basis of the models regarding the development of the caries and carry out the analysis by means of these distributions. As a step in this direction, a simple model is developed in this paper for the formation of caries in the pitted surfaces, and its validity is tested by fitting it to the data of G. and R. 1. The models for the caries on other groups of surfaces involve a larger number of parameters, and their fitting requires more efficient methods of estimating. Their study will therefore be taken up separately in a future paper.

MODEL FOR THE DISTRIBUTION OF UNILATERAL PITTED SURFACE CAVITATION COUNT

Let us assume that the number of carious surfaces in the mouth of an individual at any age t_1 depends on (1) the number of carious surfaces that he gets by "pure chance," i.e., due to influences which cannot be accounted for by simple reasoning and (2) the number of carious surfaces that he develops because he had some carious surfaces at a previous age, t_0.

Let n be the number of pitted surfaces on the side of the mouth under consideration and $p(t_0)$ the probability that a surface without caries gets affected during the interval (t_0, t_1) by pure chance. If $p(t_0)$ is small (cf. Table 1) and if m of the surfaces have no cavity at age t_0, then the probability that a new carious surface appears at age t_1 is approximately given by $mp(t_0)$, and the probability that no new carious surfaces appear by $1 - mp(t_0)$. Further, assume that the surfaces are grouped into sets of two neighboring surfaces and that, if a surface is carious, it, in essence, increases the probability of its neighbor's becoming carious by an amount, say $s(t_0)$, if the neighbor is not already carious. Then, if we denote by $P_r^{i(2),r-2i}(t)$ the probability of an individual of age t having r carious surfaces, $2i$ of which belong to i pairs of neighbors

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* In this sequel, we will be referring to this paper again and again. Hence we will abbreviate it to "G. and R. 1" from here onward.

and $r - 2i$ of which are such that none of them is a neighbor of any one of the carious surfaces, the above model leads us to the general stochastic relationship

$$P_r^{i(2),\, r-2i}(t_1) = \sum_{j=0}^{i} \binom{r-2i+j}{j} P_{r-j}^{(i-j)\,(2),\, r-2i+j}(t_0)\, s^j(t_0)\,[1-s(t_0)]^{r-2i} \times [1-(n-r+j)\,p(t_0)] + \sum_{j=0}^{i} \binom{r-2i+j-1}{j} P_{r-j-1}^{(i-j)\,(2),\, r-2i+j-1}(t_0) \times s^j(t_0)\,[1-s(t_0)]^{r-2i-1}(n-r+j+1)\,p(t_0)\,. \quad (1)$$

Here we make the convention that a term will be set equal to zero if any superscript or subscript of $p(t_0)$ is negative. Note that the first summation on the right-hand side of equation (1) refers to the probability of getting r defects when no new carious surface appears by chance during the interval (t_0, t_1) and the second summation to the probabilities when exactly one new carious surface appears by chance during the interval (t_0, t_1). The terms of equation (1) for certain small values of r are given below:

TABLE 1

FITTING OF A STOCHASTIC MODEL TO THE UNILATERAL PITTED SURFACE CAVITATION COUNT

Count per Child	Age 8 (Years)		Age 9 (Years)		Age 10 (Years)	
	Observed	Expected	Observed	Expected	Observed	Expected
0	20	20.00	12	12.00	9	9.00
1	24	24.00	18	19.00	18	18.00
2	67	66.06	46	48.48	46	43.39
3	39	32.80	40	34.38	43	46.41
4+	14	21.14	13	16.14	24	24.20
χ^2		3.58		1.66		0.41
D.F.		2		2		2
χ^2 as in G. and R. 1		14.7		11.8		9.5
D.F. for this chi-square		3		4		4
$p(t)$		0.061		0.034		0.031
$r(t)$		0.45		0.055		0.016

$$P_0^{0(2),\,0}(t_1) = P_0^{0(2),\,0}(t_0)\,[1-np(t_0)]\,,$$

$$P_1^{0(2),\,1}(t_1) = P_0^{0(2),\,0}(t_0)\,np(t_0) + P_1^{0(2),\,1}(t_0)\,[1-(n-1)\,p(t_0)]\,[1-r(t_0)]\,,$$

$$P_2^{0(2),\,1}(t_1) = P_1^{0(2),\,1}(t_0)\,(n-1)\,p(t_0)\,[1-s(t_0)] + P_2^{0(2),\,2}(t_0) \times [1-(n-2)\,p(t_0)]\,[1-s(t_0)]^2\,,$$

$$P_2^{1(2),\,0}(t_1) = P_1^{0(2),\,1}(t_0)\,s(t_0) + P_2^{1(2),\,0}(t_0)\,[1-(n-2)\,p]\,, \quad (2)$$

$$P_3^{0(2),\,3}(t_1) = P_2^{0(2),\,2}(t_0)\,[1-s(t_0)]^2\,(n-2)\,p + P_3^{0(2),\,3}(t_0) \times [1-s(t_0)]^3\,[1-(n-3)\,p(t_0)]\,,$$

$$P_3^{1(2),\,1}(t_1) = 2P_2^{0(2),\,2}s(t_0)\,[1-s(t_0)]\,[1-(n-2)\,p(t_0)] + p_2^{1(2),\,0}(n-2)\,p + P_3^{1(2),\,1}(t_0)\,[1-s(t_0)]\,[1-(n-3)\,p(t_0)]\,.$$

Obviously, the probability $P_r(t_1)$ of obtaining in all r carious surfaces is given by

$$\sum_{i=0}^{(r/2)} P_r^{i(2),\, r-2i}(t_1),$$

where $(r/2)$ stands for the largest integer less than, or equal to, $r/2$.

APPLICATION OF THE ABOVE MODEL

For illustrative purposes, this model is fitted to parts of Table VIII of G. and R. 1. In fitting such stochastic models, one is faced with two estimation problems:

1. To calculate the expected frequencies at time t_1, we need to know the values of $P_r^{i(2),\, r-2i}(t_0)$ at a previous time t_0. In the data, the values corresponding to $P_r^{i(2),\, r-2i}(t)$ are generally so small—and hence highly susceptible to random fluctuations—that regarding the sample values as the true values for some t introduces inefficiency in the evaluation of the distribution for subsequent years. Hence the distribution needs to be estimated for some basal year too.

2. The quantities $p(t)$ and $s(t)$ also need to be estimated.

As for equation (1), we first assume that the sample at age six represents the distribution at age six exactly, get the expressions for the first fair frequencies at age seven by using equations (2), and equate these to the observed values at that age. For n, we choose the values from Table IX of G. and R. 1.* Then we have

$$\begin{aligned}
P_0(7) &= \tfrac{30}{156} = \tfrac{31}{94}[1 - 5p(6)], \\
P_1(7) &= \tfrac{31}{156} = \tfrac{31}{94} 5p(6) + \tfrac{28}{94}[1 - 4p(6)][1 - s(6)], \\
P_2(7) &= \tfrac{62}{156} = P_2^{0(2),\,2}(7) + P_2^{1(2),\,0}(7) \\
&= \tfrac{28}{94}[1 - s(6)]\, 4p(6) + P_2^{0(2),\,1}(6)[1 - s(6)]^2[1 - 3p(6)] \\
&\qquad + \tfrac{28}{94} s(6) + P_2^{1(2),\,0}[1 - 3p(6)], \qquad (3) \\
P_3(7) &= \tfrac{30}{156} = P_3^{0(2),\,3}(7) + P_3^{1(2),\,1}(7) \\
&= P_2^{0(2),\,2}(6)[1 - s(6)]^2 3p(6) + P_3^{0(2),\,3}(6)[1 - s(6)]^3 \\
&\qquad \times [1 - 2p(6)] + 2P_2^{0(2),\,2}(6)\, s(6)[1 - s(6)][1 - 3p(6)] \\
&\qquad + P_3^{1(2),\,1}(6)[1 - s(6)][1 - 2p(6)].
\end{aligned}$$

Upon using the fact that

$$P_2(6) = P_2^{0(2),\,2}(6) + P_2^{1(2),\,0}(6) = \tfrac{23}{94} \qquad (4)$$

and

$$P_3(6) = P_3^{0(2),\,3}(6) + P_3^{1(2),\,1}(6) = \tfrac{10}{94}, \qquad (5)$$

* In general, we propose that records be made of unilateral pitted surfaces of each individual and take their average as an estimate of n. This will avoid some of the loss of power that occurs when one bases his judgment about n on the sample distribution under consideration.

we can solve equations (3) to get estimates of $p(6)$, $s(6)$, and $P_r^{i(2),\,r-2i}(6)$ for $i = 0, 1, \ldots, (r/2)$ and $r = 0, 1, 2, 3$. Hence $P_r^{i(2),\,r-2i}(6)$ can be calculated for $i = 0, 1, \ldots, (r/2)$ and $r = 0, 1, 2, 3$. Since the sample value of $P_4(7)$ is very small $(= 3/156)$, we set $P_4^{2(2),\,0} = 3/156$ and all other $P_4^{i(2),\,4-2i}(7)$ equal to zero. Of course, the $P_r^{i(2),\,r-2i}(7)$'s are zero for $r > 4$. Having thus gotten the detailed distribution at age seven, we now compute the expected frequencies at age eight. The only unknowns at this stage—namely, $p(7)$ and $s(7)$—are estimated by using the first two frequencies at age eight, since we know of no better methods. The expected frequencies are given in Table 1, third column.

It is to be noted that the estimation of $P_r^{i(2),\,r-2i}(7)$ is not based on the sample at age eight. Since the hypothesis to be tested is regarding the change of the distribution, the estimation implies that the tabulated χ^2 is an overestimate of the true χ^2 that one would get if the hypothesis were correct and were being tested without introducing any errors. The true χ^2 would have the large-sample Pearson chi-square distribution with $5 - 2 - 1 = 2$ degrees of freedom, 5 being the number of classes, 2 being subtracted for having estimated two parameters and 1 being subtracted to account for the fact that the total of the frequencies adds up to the total number of children, which is a fixed quantity.

Now the expected frequencies at age seven can be calculated from those at age eight by using equations (2); but, since the expected frequencies at age eight themselves were based on the estimated and not the exact distribution at age seven, this procedure will lead to high inefficiency. Also, we cannot use a method analogous to the one above of taking help of the samples at ages seven and eight, since, first, $P_4^{i(2),\,r-2i}(8)$ are not estimable and, second, they are not too small to be neglected. Hence we propose the following method:

Take the observed proportion $\hat{\hat{P}}_r(8)$ of count r at age eight and get the new estimate of $P_r^{i(2),\,r-2i}(8)$ by using the formula

$$\hat{\hat{P}}_r^{i(2),\,r+2i}(8) = \frac{\hat{P}_r^{i(2),\,r-2i}(8)}{\hat{P}_r(8)}\,\hat{\hat{P}}_r(8), \qquad (6)$$

where $\hat{P}_r^{i(2),\,r-2i}(8)$ in equation (6) is the value of $P_r^{i(2),\,r-2i}(8)$ as calculated above. The expected frequencies obtained by this procedure are given in Table 1, fifth column. Degrees of freedom are calculated along the same lines as in the above example.

Observe that even the tabulated values of chi-square for these fits are very much smaller than those given by G. and R. This indicates that the true model underlying the population is close to the model given above.

The expected frequencies obtained by using the same procedure are given for age ten in Table 1, seventh column. The small chi-square—while asserting the above statements—gives faith that the *ad hoc* method of estimation used in the process is not too inefficient.

POSSIBLE EXTENSIONS OF THE ABOVE MODEL

An immediate extension of the above model is to consider the teeth of an individual as comprised of m disjoint sets of four teeth each. For example, a typical set may consist of a tooth on the left side, its counterpart on the right-hand side, and the two teeth opposite to them. In this case a set can be in one of the following seven phases:

(1) no teeth carious, (2) only one tooth carious, (3) a tooth and its counterpart carious, (4) a tooth and its opposite carious, (5) a tooth and the one opposite to its counterpart carious, (6) any three teeth carious, and (7) all four teeth carious. The individuals with r caries are divided into subclasses, one corresponding to each of the possible vectors of the form $r(1), r(2), \ldots, r(7)$, where $r(i)$ refers to the number of sets in the ith phase. It is easy to see that we can develop by assuming that the decaying in one set in no way affects the decaying in other sets and that not more than one tooth decays in the time interval (t_0, t_1). An example where such a model may be applicable is the distribution of the (bilateral) pitted surface cavitation counts (Table V of G. and R. 1). It is obvious that, in fitting such extended models, a knowledge of the observed frequencies in every subclass mentioned above is essential, at least for the basal year. Estimation of parameters and other aspects of fitting are similar to those of the case discussed in the earlier section. Detailed discussion is omitted for brevity.

CONCLUSION

Distributions such as the negative binomial fit a large amount of data well, partly because of the flexibility of shape and partly because a heterogeneous population approximates closely to distributions like the Pascal, which are obtainable by superimposing a number of Poisson distributions with different means. The author believes that much of the good fits obtained by G. and R. 1 is due to this reason. The fact that the Pascal distribution does not represent the distribution of caries at large becomes apparent when it is noted that the Pascal distribution has a mean always smaller than the variance, while the distribution of caries will have high mean and near-zero variance for people advanced in years.

On the basis of the relatively good fits with our model in Table 1 in contrast with the ones with the negative binomial, the following conclusions are drawn: (1) caries are highly contagious, and (2) pitted surfaces at a given age are very homogeneous in their susceptibility. The decreasing values of $p(t)$ and $s(t)$ (cf. Table 1) show that the teeth acquire strength with age and that their susceptibility to decay decreases. In conclusion, it is observed that the large number of carious surfaces in the mouth of an elderly person is due to the fact that the decayed teeth do not heal.

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Gingival-Bone Count: A Method for Epidemiological Study of Periodontal Disease

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The epidemiology of periodontal disease is one of the important challenges before the dental profession at the moment, but it has been much hampered by the lack of quantitative measurements that are reasonably accurate and yet simple enough to be applied to large populations without excessive expenditures of time or the likelihood of wide interpretive differences between observers. There are a number of obstacles in the path of the study of periodontal disease, among which the following are the most important:

1. Two areas are involved in most instances: the gingiva, where gingivitis is usually a reversible process, easy to observe; and the alveolus, where bone loss is the best measure of damage and is almost always chronic, cumulative, and hard to observe.

2. Periodontal disease, unlike caries, has its greatest incidence late in life. A patient seen at this age will probably have lost teeth for reasons other than periodontal disease. This means that valid assumptions are usually impossible as to the reason or reasons for the loss of teeth which are found missing at the time of examination. DMF counts of children's teeth can be interpreted with reasonable accuracy on the assumption that the missing teeth were lost because of caries. Except in longitudinal studies, however, measures of periodontal disease must usually be made without including missing teeth. This involves the assumption that the missing teeth were affected by periodontal disease to the same degree as those still present in the mouth—an assumption quite possibly erroneous but the best we can make under the circumstances.

3. Alveolar bone loss is very difficult to evaluate clinically without the use of the X-ray. Observational errors as to pocket depth accumulate easily, with a bias toward underestimation, and interpretive differences are wide because estimates of tooth mobility and impairment of function, as well as degree of bone loss, may vary widely.

The measures which have evolved in this field are of several types. Some measures, like Massler and Schour's PMA index,[1] deal with gingival conditions alone. Others, such as Sheppard's,[2] deal solely with bone resorption as seen by X-ray. A third group combines gingival and bone conditions into a single figure in which the relationship between the two tissues is lost to sight. Two of the most interesting recent methods, Russell's Index[3] and Sandler and Stahl's Periodontal Disease Rate,[4] fall into this category. A method is needed that will provide not only an over-all score for the individual patient but also some information on the relationship between the two major disease processes that combine to make up this score. The success of the DMF tooth count, which can be so easily separated into its original components of decayed, missing, and filled teeth, indicates that such a "two-in-one" type of measurement is possible. The

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DMF count, however, is an index in which the components are mutually exclusive and exhaustive of conditions signifying dental caries experience. The concept, although very desirable, is more elusive in the field of periodontal disease.

The measurement of gingivitis has almost always been subjective. There are no valid criteria for objective measurement of gingivitis at any given point. The PMA index merely combines subjective measurements in different areas around a tooth. Subjective assessment, where numbers are needed, makes use of a small number of categories, never apparently above five, arranged in order of severity. One of the earliest such measures and one of the best known is the system of four plus signs by which Wassermann tests are described. These pluses and a zero make five categories, beyond which it is difficult for reliable subjective observation of degree or intensity to be made in most biological matters. Day and Shourie[5] use four categories (zero to 3+) in the measurement of gingival disease. Similarly Greene, in his Oral Hygiene Index,[6] uses scores of zero to 3, for both debris and calculus.

The evaluation of bone loss presents a difficult problem. Simple linear measurements are possible here, such as millimeters of bone loss, but, even where X-rays are available, this method has disadvantages. The "normal" position of the alveolar crest is usually estimated in relation to the dentoenamel junctions of the teeth either side, but this position is hard to define, since tilting of teeth in their sockets and many other factors make for quite permissible variations in the normal. Elongation or foreshortening of teeth in roentgenograms also makes accurate measurement in many instances impossible, as does alveolar bone loss on the buccal or lingual side of the root. Where one measurement only is to be made upon a tooth, it is hard to know how to handle a pocket on one proximal surface not matched by a pocket on the other side. Finally, the damage to a tooth from bone loss is not in proportion to actual millimeters but in proportion to the fraction of its root area that has been denuded. One millimeter bone loss means more on a short root than on a long one. For these reasons, it would appear that the epidemiologist cannot rely upon linear measurements alone, even in the field of bone loss, and will do better to rely instead upon his estimate of the proportion of root denuded or function lost.

Sheppard's measurement grades the proportion of bone resorption from zero to 10. Ten denotes complete loss of alveolar bone as far as the root apex; 5 indicates loss of half the alveolar support; and 1 denotes just sufficient bone loss to be visible. Day and Shourie used this measure in a large study of periodontal disease in India.[7] Evaluation of a single tooth, however, by a system involving eleven categories becomes very complex. A simpler method is important, and Russell's Index goes a long way in the direction of such simplification. The scores here range from zero to 8. Scores of zero through 2 are used to measure gingivitis with "radiographic appearance—essentially normal." A score of 4 is used where there is "early notch-like resorption of the alveolar crest." Beyond this, four numbers are available for increasing degrees of periodontoclasia, though apparently numbers 5 and 7 are not in practical use. Number 6 is used to describe "gingivitis with pocket formation." It is to be assumed, therefore, that the degree of gingivitis has something to do with the assignment of numerical scores throughout the entire series. The scores are arranged to denote order of severity, but the process of averaging them for a whole-mouth score forces a concept that they are in arithmetic progression and also blends the two disease processes indistinguishably.

For sheer simplicity, Sandler and Stahl's Periodontal Disease Rate has much to commend it. Each individual tooth present in the mouth is here rated as to the existence or absence of periodontal disease. There is no differentiation as to the character of the lesion, its chronicity, the degree of bone loss, or the prognosis. The authors demonstrate a similarity between measurements made by this rate and by the Russell Index. This is helpful, just as is the curve by which Knutson measures the relationship between proportion of population showing some evidence of caries and the number of DMF teeth per person in that population.[8] Knutson's curve, however, is of little value in predicting a DMF count from a caries prevalence above, say, 85 per cent. A very small variation in prevalence makes a large variation in the DMF count. It is known also that the standard deviations of observed DMF counts are greater as the counts themselves increase,[9] thus compounding any error at the high end of the scale. Similarly, in cases of advanced periodontal disease it is easy to imagine the Periodontal Disease Rate approaching or even reaching 100 per cent without giving much-needed discrimination between degrees of periodontoclasia. Indeed, Sandler and Stahl's scatter diagram of the relationship between Russell's Index and the Periodontal Disease Rate in 100 patients (their Fig. 1) shows the eleven cases with 1.0 values for PDR to have Russell Index values ranging all the way from 4.0 to an apparent 7.9.

In careful epidemiological study, more detail would be desirable than that provided either by the Periodontal Disease Rate of Sandler and Stahl or by the Russell Index. The method must not be too complex, however, or cost and training factors will preclude examination of large enough samples. Clinical approximations of proportion of root length denuded must almost undoubtedly be used, nor are they likely to be much less accurate than results obtained by more elaborate quantitative measurements.

EXPERIMENTAL METHODS

Index.—Table 1 describes a new index to permit differential recording of gingival and bone conditions. Subjective measurement of gingivitis is made on a zero–3 scale for each tooth, and proportional measurement of bone loss is made on a zero–5 scale. Whole-mouth mean scores are then added together to obtain what could be called a "GB count." This count weights gingivitis and bone loss on a 3–5 basis: an arbitrary relationship, realistic only to the extent that bone loss makes a greater threat to the usefulness of the tooth than gingivitis. As the whole-mouth means for gingivae and bone are separately recorded, the conditions making up the GB count can always be known. No attempt is made to evaluate the missing teeth in the mouth.

The scoring system used is applicable whether explorer examination, bite-wing X-ray examination, full X-ray survey, or a combination of these methods is used. A preliminary test of the method makes it obvious that the differences between clinical mean scores and mean scores obtained by the use of explorer examination plus X-ray, at least in early cases of periodontal disease, are greater than would be found in caries examining. One pair of bite-wing X-rays under test conditions[10] only increased DMF explorer findings 4–18 per cent, while a similar pair of pictures in the present study increased a clinical GB count for 29 cases by 21 per cent. The X-ray bone scores *per tooth observed* were *five times* as high as the clinical bone scores per tooth observed.

An explorer or pocket probe will do fairly well in locating specific pockets, but overall bone loss is extremely difficult to estimate by clinical methods alone. It is likely,

therefore, that field survey work will rely much more on simple X-ray procedures (probably the use of two posterior bite-wing X-rays) than does current field surveying for caries. Experience will settle this point, but, whatever the answer, group gingival or bone scores made by a given method can be compared with other group scores made by the same method, just as is the case in DMF survey work. It is only necessary to record accurately the criteria that were used in examination.

If the prevalence of individuals with periodontal disease is desired, it is only necessary to compare those cases showing a GB count of zero with those cases showing GB counts above zero.

The index in its present form does not distinguish between slight involvement of many teeth and extensive involvement of a few teeth. If information as to type of bone resorption is desired, additional "typing" of cases will be needed, as was done by Day and Shourie.[5]

TABLE 1

SCORING SYSTEM FOR GB COUNT

Gingival score (one score is assigned to each tooth studied. A mean is then computed for the whole mouth).	
Negative	0
Mild gingivitis involving the free gingiva (margin, papilla, or both)	1
Moderate gingivitis involving both free and attached gingiva	2
Severe gingivitis with hypertrophy and easy hemorrhage	3
Bone score (one score is assigned to each tooth studied. A mean is then computed for the whole mouth).	
No bone loss	0
Incipient bone loss or notching of alveolar crest	1
Bone loss approximating one-fourth of root length or pocket formation one side not over one-half root length	2
Bone loss approximating one-half of root length or pocket formation one side not over three-fourths root length; mobility slight*	3
Bone loss approximating three-fourths of root length or pocket formation one side to apex; mobility moderate*	4
Bone loss complete; mobility marked*	5
Maximum possible GB count per person	8

* If mobility or impairment of masticatory function varies considerably from that to be expected with bone loss seen, the score may be altered up or down one point.

Test on 108 cases.—An easily available sample for testing the new index was found in a group of 108 students from Harvard and Radcliffe colleges. Most of the sample was composed of young people with no more than incipient periodontal disease, but a few graduate and foreign students were included whose periodontal problems were more advanced. The sample was assembled by picking cases with some degree of bone loss from the entire series of No. 3 posterior bite-wing films taken for the entering students at both colleges as part of their entrance physical examinations. Since these X-rays were available from the start, the survey has been based upon a combination of clinical and X-ray findings. In 29 of the cases, however, careful clinical examination was performed before reference to the X-rays, and a separate scoring of bone loss was made, to estimate the value of the clinical method alone in this matter. All cases were given a bone-loss recording by X-ray alone, the object of this being to assess the value of a survey where X-rays could be taken but clinical examination was impossible. Gingival conditions were appraised by clinical examination alone, as would, of course, be necessary. Figure 1 shows the recording of a typical case from the sample. Table 1 gives the definitions of the scores used.

RESULTS

The findings of the study are found in Table 2, the columns of which may be described in order as follows:

The gingival score (Gc) was ascertained clinically by mirror and No. 17 explorer. The mean value for the group was 1.12, with a standard deviation of 0.70.

The clinical bone score (Bc) was obtained by probing with an explorer to determine the amount of bone loss surrounding each tooth. The mean value for the 29 cases in

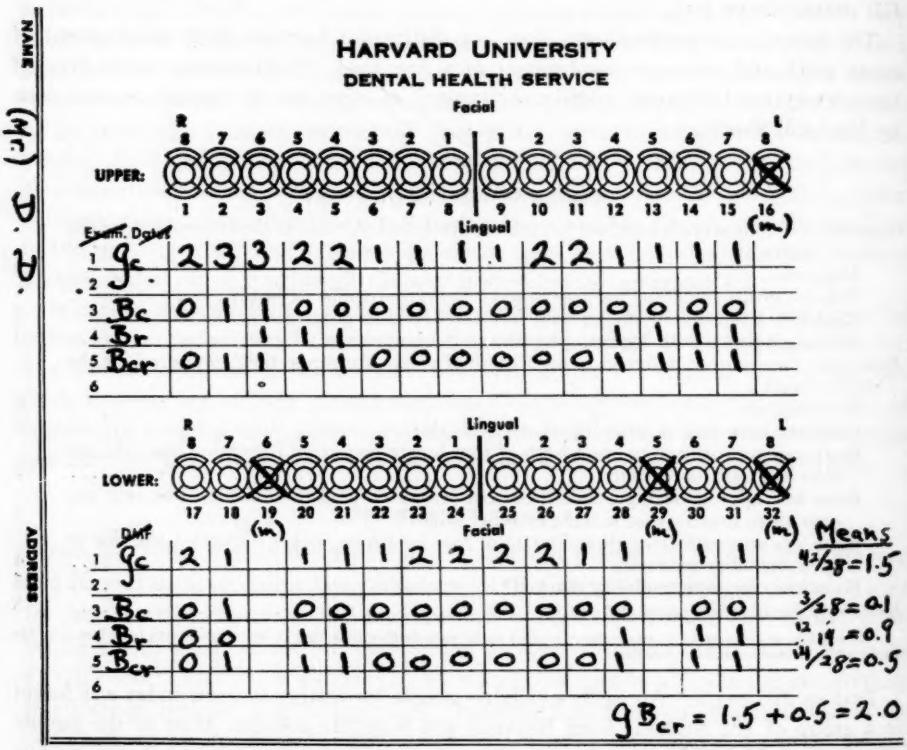

FIG. 1.—Clinical examination record of gingivitis and bone loss for a typical case in the sample. See Table 2 for explanation of symbols.

which this process was performed separately was 0.18. The standard deviation was 0.22. On the average, 28.2 teeth were studied clinically in each case.

The X-ray bone score (Br) was based upon findings from two No. 3 posterior bite-wing X-rays. The mean score for the group was obtained by dividing the sum of the scores by the *number of teeth seen in the films*—not all teeth present in the mouth. This mean score was 0.95, with a standard deviation of 0.47. On the average, 17.3 teeth were studied by X-ray in each case.

The X-ray bone score was combined with clinical findings made by explorer. This combined score (Bcr) was 0.67 for the group and carried a standard deviation of 0.42.

The gingival score (Gc) was then added to the combined clinical and bite-wing

X-ray bone score (Bcr) to give the GB count (GBcr). The mean for the group was 1.79, and the standard deviation was 1.03.

Whole-mouth mean scores will be seen to have been carried to one decimal place for an individual and to two decimal places for the group. Using the second, fifth, and sixth columns, one would say that this group had a G score of 1.12, a B score of 0.67, and a GB count of 1.79. All examinations and recorded scores are the work of one person (L. B. L.). The X-ray bone scores (Br) and clinical bone scores (Bc) correlated to a level of $r = 0.96$. When independent readings of 31 of the X-ray surveys were made by the other person (J. M. D.), the two sets of X-ray readings on these cases correlated to a level of $r = 0.88$. Further joint work would probably have increased this correlation.

TABLE 2

FREQUENCIES OF WHOLE-MOUTH MEAN SCORES

Score	Gingivae Clinical (Gc)	Bone Clinical (Bc)	Bone Bite-Wing X-Ray (Br)	Bone Clinical and X-Ray (Bcr)	GB Count Clinical and X-Ray (GBcr)
0.0–0.2	8	23	1	3	0
0.3–0.5	14	5	12	51	4
0.6–0.8	24	0	36	35	10
0.9–1.1	19	1	40	9	16
1.2–1.4	15	0	9	4	18
1.5–1.7	8	0	2	1	16
1.8–2.0	7	0	3	2	15
2.1–2.3	4	0	2	2	7
2.4–2.6	3	0	2	0	3
2.7–2.9	6	0	1	0	5
3.0–3.2	0	0	0	1	4
3.3–3.5	0	0	0	0	3
3.6–3.8	0	0	0	0	3
3.9–4.1	0	0	0	0	0
4.2–4.4	0	0	0	0	0
4.5–4.7	0	0	0	0	2
4.9–5.1	0	0	0	0	1
5.2–5.4	0	0	0	0	0
5.5–5.7	0	0	0	0	0
5.8–6.0	0	0	0	0	0
6.1–6.3	0	0	0	0	1
Total	108	29	108	108	108
Mean	1.12	0.18	0.95	0.67	1.79
Standard deviation	0.70	0.22	0.47	0.42	1.03

DISCUSSION

Each of the score ranges (zero to 3 for gingival conditions and zero to 5 for bone conditions) offers a scale for subjective estimates which should be simple enough to be reliable in the hands of the average observer. The bone score, where some estimate can be made of the fraction of a tooth root which is denuded, has more divsions in it than the gingival score. It is a fortunate accident that these two scoring systems give top values which relate to one another in a manner (3:5) indicative of the seriousness of the conditions they describe. Bone loss is a more serious threat to a tooth than is gingivitis, but both are almost always present when the tooth must be extracted. An adding of the scores to form a composite count, though arbitrary, may be considered

permissible. Regardless of one's agreement with this concept, however, it is always possible to separate a GB count into its original gingival and bone components—more than can be done with any other index presently available.

Some observations of interest may be made upon the findings of the survey of 108 cases.

1. The mean gingival score is higher than that for bone. This may be due to an artifact of the scoring system but is more likely to be due to the type of population employed in this investigation—a young population with only incipient bone loss but greater gingival involvement. There is no significant difference between the prevalence of zero gingival scores (Gc) and the prevalence of zero bone scores (Bcr).

2. The mean clinical bone score (Bc) is only one-fourth to one-sixth that obtained by X-ray (Bcr or Br). This is due to the difficulty in determining the loss of interproximal alveolar bone by explorer alone. Only advanced cases of bone loss can be picked up with certainty by explorer. A full-mouth X-ray survey taken for each member of the group would almost undoubtedly have given an even higher mean bone score than did the posterior bite-wings.

3. The X-ray bone score (Br) is higher than the combined clinical X-ray bone score (Bcr). This is due to the fact that the latter is a composite whole-mouth score, with explorer findings the only ones available for the anterior teeth but with the score raised above the level of a complete clinical score by the inclusion of posterior X-ray findings.

4. The standard deviations are all quite high. Coefficients of variation (standard deviation/mean $\times$ 100) are not unlike those for DMF counts for similar populations (Dunning, Table III),[9] but clinical impression suggests a greater range in variability in periodontal conditions than is associated with dental caries, as age increases.

5. When a coefficient of correlation was calculated between the bone findings as determined by bite-wing X-rays alone and those by bite-wing X-ray plus clinical examination, a value of $r = 0.909$ was obtained. This high correlation suggests that reliable estimation of bone loss can be obtained by bite-wing X-ray examination alone. Neither clinical nor posterior bite-wing examination nor a combination of the two, however, constitutes a full periodontal examination. Each is a standardized, if incomplete, measure, but the bite-wing method is far better than clinical examination alone.

The need for further work is obvious. Field study under a variety of conditions will be needed in order to test the practicality of the index and its discriminative value. Studies should also be made in which full-mouth X-rays are taken, in order to indicate the proportion of the whole periodontal story that can be learned from clinical examination alone or from clinical examination plus posterior bite-wing X-ray.

SUMMARY

A new index is presented in which simple subjective measurements of gingival condition can be combined with simple proportional measurements of bone loss in order to produce a composite score, the original components of which can be identified. The term "GB count" is used to describe this composite score. It is felt that the method will prove more descriptive in epidemiological work than any of the existing methods that combine gingival and bone conditions indiscriminately.

A trial study of the method has been carried out on Harvard and Radcliffe students

in whom gingival disease was not uncommon but bone loss was, for the most part, incipient. The GB count found was 1.79 out of a total possible score of 8. This figure was composed of scores of 1.12 for gingival disease out of a possible score of 3 and 0.67 for bone loss out of a possible score of 5.

Large standard deviations appeared in all measurements as a result of the high variability of periodontal disease. There was also a great difference between mean values obtained by clinical examination alone, those obtained by bite-wing X-ray alone, and those obtained by clinical plus bite-wing examination. However, when bone scores determined by bite-wing X-rays plus clinical examination were paired with bone scores by bite-wing alone, a high correlation coefficient (0.91) resulted.

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Estimation of Arsenic in Teeth by Activation Analysis

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From time to time various analytical methods have been applied to tooth substance to establish the content of so-called trace elements such as lead and tin.[1, 2] Other experimental work has shown that these trace elements may play an important part in the formation and preservation of teeth.[3-6] As trace elements are present in very low concentrations, it has been necessary in previous investigations to use pooled material from many different subjects to provide an adequate sample for analysis by chemical, spectrographic, or colorimetric methods. The conclusions which may be drawn from such experiments are necessarily limited by the non-specific nature of the material.

Radioactivation analysis is a technic by which many elements may be detected and estimated with a greater sensitivity than that of conventional analytical procedures. The essential basis of the method is that the element to be determined is made radioactive by exposing the sample to bombardment by neutrons inside a neutron reactor. The radioactivity induced in this way has properties which are characteristic of the element concerned. After suitable chemical separation of the element, if necessary, the amount of the induced radioactivity, the rate of its decay, and the energy of the associated nuclear radiations can be measured without much difficulty by instruments such as Geiger and scintillation counters.

When the only stable isotope of arsenic is irradiated with thermal neutrons, an unstable isotope is produced by neutron capture. This unstable isotope decays with a half-life of 26.8 hours, with the emission of β-particles and γ-rays. The activation cross-section is 4.0 barns, and the saturation activity for a pile flux of 10^{12} neutrons per square centimeter per second is about 2×10^{12} disintegrations per minute per gram of elemental arsenic. The reaction is represented by

$$As_{33}^{75} + n_0^1 \rightarrow As_{33}^{76}.$$

The sensitivity of the determination is 10^{-9} gm. compared with the highest chemical sensitivity of 10^{-7}–10^{-8} gm.

Submicrochemical separations are avoided when inactive carrier arsenic is added to increase the total weight of arsenic present. In addition, "carriers" may be added to hold back other active atoms produced. The accuracy of the determination is limited only by the statistical error of counting.

EXPERIMENTAL METHODS

Longitudinal ground sections of 25 sound permanent teeth were prepared to a thickness of 400 μ. Samples of these teeth containing both enamel and dentin and weighing not more than 10 mg. were taken.

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About 3–6 mg. of the tooth sample were weighed and sealed in a polythene bag. One or 2 mg. of a sample of arsenious oxide were also prepared in this way for use as a standard. The weights were accurate to $^1/_{100}$ mg.

The polythene bags containing the samples were placed in a standard aluminum irradiation can and sent to a suitable atomic pile for irradiation by low-velocity neutrons. The can remained in the pile for 24 hours, when the activity generated was sufficient to be used for analysis. The standard sample was removed, dissolved in sodium hydroxide solution, and made up to 1 liter. One milliliter of this solution was taken and made up to 100 ml. One milliliter of this solution was taken as the standard. From this it was possible to calculate the count rate expressed as counts per minute per milligram of elemental arsenic. The method of separation of the arsenic was a modification of Thomas and Collier's Gutzeit technique[7] and was carried out as follows:

The tooth sample was removed from the bag and digested in a mixture of 3 ml. of concentrated sulfuric acid and 5 ml. of concentrated nitric acid and heated until all the nitric acid had been removed. The remaining acid with the arsenic retained in it was well washed in a 200-ml. flask. A further 2 ml. of concentrated sulfuric acid and 4 ml. of concentrated hydrochloric acid were added, together with 10 μg of inactive arsenic as a carrier, followed by 5 ml. of a 15 per cent solution of sodium iodide and 0.4 ml. of a 40 per cent solution of stannous chloride in 50 per cent hydrochloric acid. The solution in the flask was then diluted to about 150 ml., and the flask was placed in a boiling-water bath for 5 minutes. Ten grams of 16–22-mesh zinc pellets were added, and the reaction was allowed to continue for 15 minutes. During this reaction the arsenic was evolved as arsine. Hydrogen was also evolved and, together with the arsine, was passed through a cotton-wool filter impregnated with lead acetate to remove any small amounts of hydrogen sulfide liberated from the reaction mixture. The arsenic in the form of arsine was removed from the hydrogen by passing it through a trap containing 1 ml. of 1.6 per cent solution of mercuric chloride. Five milliliters of a 0.001 N iodine solution in 40 per cent sodium iodide were then added to the mercuric chloride to complete the solution of any solids formed. The solution was then made up to a standard volume, and the activity was estimated by using a Geiger-Müller counter. This was compared with the standard sample and the arsenic content so obtained. As the presence of antimony could be a possible source of error in the determination, decay curves were prepared from the samples of arsenic separated from the teeth. These curves showed only the presence of As.[76]

RESULTS

The results of the analysis of 25 sound permanent teeth showed the arsenic content of normal teeth to lie within the range of 0.031–0.145 ppm, giving a mean value of arsenic for sound human teeth of 0.060 ppm.

SUMMARY

The activation analysis method described is a quick and accurate technic for the estimation of arsenic in biological materials. After nitric/sulfuric acid digestion of the activated sample, the Gutzeit separation is used in conjunction with an estimation by using a Geiger tube accepting liquid samples. The method has the advantage that

very small samples can be tested without difficulty. Using this method, the arsenic content of 25 teeth was estimated, and a mean value of 0.060 ppm was obtained.

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Studies on the Permeability of the Oral Mucosa
I. Permeability of Inorganic Ions

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It has been reported that various chemical substances permeate the oral mucosa by simple diffusion.[1-5] However, the basic physiologic mechanism of this phenomenon has not yet been confirmed experimentally. Recently, many basic problems concerning the mechanism of ionic transport through biological membranes have been elucidated experimentally and theoretically.[6-10] Most of these studies have been concerned only with monolayer membranes; hence the results may not be applicable to oral mucosa.

In this experiment the permeability of K, Na, and Ca ions across the oral mucosa was investigated in order to understand the principal mechanism of ionic transport across the tissue membrane.

MATERIALS AND METHODS

The sublingual mucosa of the dog was used throughout the experiment. The procedure of removing the membrane was carried out under anesthesia,* and the mucosa was carefully excised from the submucosa and washed with physiological saline solution. As shown in Figure 1, two lengths of polyethylene tubing of different diameters (6 and 8 mm.) were connected so as to form a U-shape. A piece of fresh membrane (3 cm. in diameter) was set at the joint between the two tubes, the outer side (mucous surface) of the membrane facing the outer tube, and the inner side facing the inner tube.

After the membrane was set, it was washed several times with 0.9 per cent NaCl solution for 2–3 minutes to remove completely any blood adhering to the surface. This treated membrane was used throughout the experiment to measure the ion flux across the membrane. Both tubes were then filled with physiological saline solution and kept at 30° C. in a thermostatic bath for about 10 minutes. This saline solution was then poured off, and fresh physiological saline solution was poured into the inner tube and the experimental solution into the outer tube.

Estimations of the K ion flux across the mucosa were obtained with the use of KCl dissolved in 0.9 per cent NaCl solution. For estimating the Na ion flux, NaCl was dissolved in potassium phosphate buffer (pH 7.0). In addition, the flux of the Ca ion was measured with either $CaCl_2$ or $Ca(H_2PO_4)_2$ dissolved in 0.9 per cent NaCl solution. To avoid a difference in hydrostatic pressure, the surface level of the solutions in both tubes was kept constant, the experimental solution in the outer tube being

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* Amipansoda, manufacured by Shionogi Co., Ltd.

5 ml. and the volume of the saline solution in the inner tube being 3 ml. Measurement of the inorganic ions was carried out every 10 or 20 minutes in each experiment.

The oral mucosa was kept under an ionic gradient for a definite time, after which the experimental solution was taken from the inner tube. This solution (3 ml.) was hydrolyzed with *n*-HCl and ashed at 500°–700° C. for 30 minutes, and 3 ml. of distilled water were added to this sample. A flame photometer* was used for the measure-

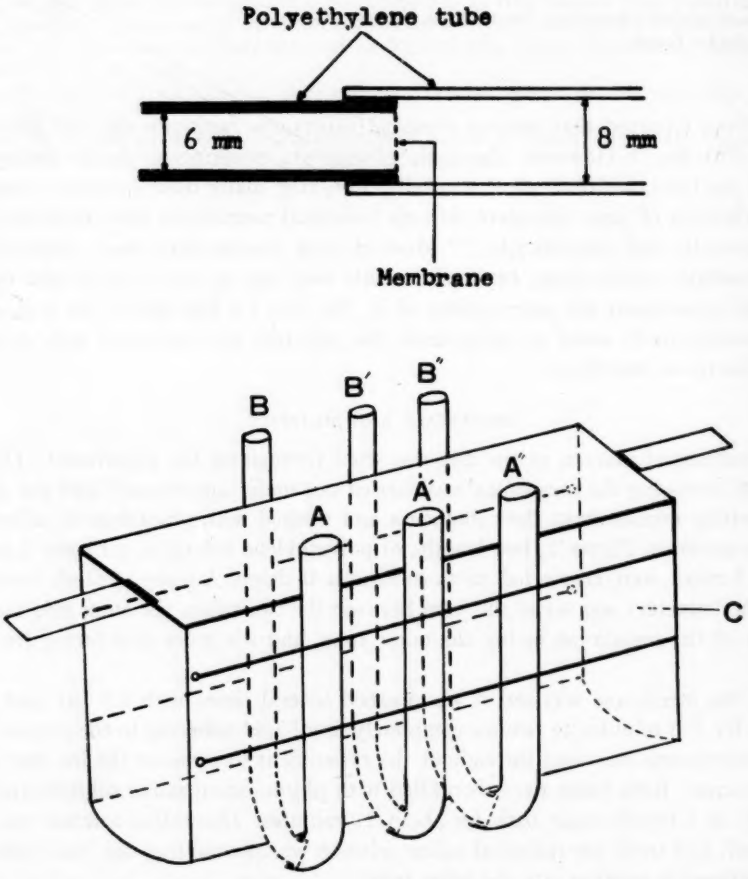

FIG. 1.—Experimental apparatus. *A–B*, *A′–B′*, and *A″–B″* = polyethylene tube; *C* = wooden block

ment of K, Na, and Ca ions. The wave length for the K ion was 768 mμ, for the Na ion it was 589 mμ, and for the Ca ion it was 423 mμ. After the first measurement, fresh solutions were used for each successive measurement.

As a preliminary step, to estimate the vitality of the excised mucous membrane, the time processes of oxygen uptake were estimated by means of the Warburg manometer. The slice of testing oral mucosa was kept in a 0.9 per cent NaCl solution and a potassium phosphate buffer.

* Beckmann.

RESULTS

As shown in Figure 2, it was clear that the oxygen uptake of the slice was constant for about 120 minutes, and the Q_{O_2} was almost the same value in either 0.9 per cent NaCl solution or potassium phosphate buffer. This result indicated that a piece of excised oral mucous membrane has a physiologic activity for about 120 minutes. Therefore, the following experiments were performed within this period.

1. *Permeability of potassium across the membrane.*—When a physiological saline solution was applied in both tubes for 20 minutes at 30° C. without the addition of the K ion, about 10 r/ml of the K ion were detected from the solution in the inner tube. This fact indicates that under this experimental condition there is a spontaneous migration of K ions from the inner side of the membrane itself. Therefore, before the main experiment, it was necessary to determine the rate at which K ions migrated from this membrane. The rate of spontaneous migration of K ions from the membrane into the inner tube is shown in Figure 3. In this figure, black and white histograms show different samples under the same experimental conditions.

When the spontaneous migration rate was approximately 15 r/ml/20 minutes, an average value of about 10 $r/(0.3)^2\pi d/20$ minutes of K ion migrated from the oral mucosa (0.3 being the radius of the 6-mm. tube and d the thickness of the membrane).

When a solution of 10^{-2} M concentration of potassium chloride was applied, the net flux of K ions was recognized to be 32 r/ml/20 minutes, and with a concentration of 10^{-3} M it was about 27–30 r/ml/20 minutes. The rates of the flux were obtained by subtracting the rates of spontaneous migration of this ion from the total potassium volume of the inner tube. That is, the rate of net flux of the K ion was changed in proportion to the concentration of potassium chloride in the outer tube. As shown in Table 1, the rate of net flux of the K ion was 44 r/ml at 40 minutes and 82 r/ml at 60 minutes from the beginning of the experiment.

After 80 minutes, K ions continued to migrate for about 10–20 minutes, even after removing the potassium solution from the outer tube. This fact suggests that K ions accumulated in the membrane during this experimental procedure.

2. *Permeability of sodium across the membrane.*—The relationship between the concentration of sodium ion in the outer tube and the rate of Na flux into the inner tube was almost the same as in the case of the potassium ion described above. When 5×10^{-3} M of sodium chloride was applied in the outer tube, the rate of net flux of Na ions was about 45 r/ml/20 minutes. In the case of 10^{-2} M of NaCl, the rate of net flux was about 50 r/ml/20 minutes. The rate of Na flux into the inner tube increased with greater concentration of sodium ions in the outer tube.

Spontaneous migration of the Na ions into the inner tube was also observed at a rate of about 10–15 r/ml/20 minutes. As shown in Figure 4, the rates of net flux of Na ions across the membrane decreased with time. It is assumed, therefore, that some differences exist between the processes of flux of K ions and Na ions across the membrane.

The effect of the existence of divalent cations on the flux of monovalent cations was analyzed, in order to learn the basic mechanism of ionic permeability of tissue membrane. As shown in Table 2, the net flux of potassium ions through the membrane was not affected when Ca ions were present in the outer tube.

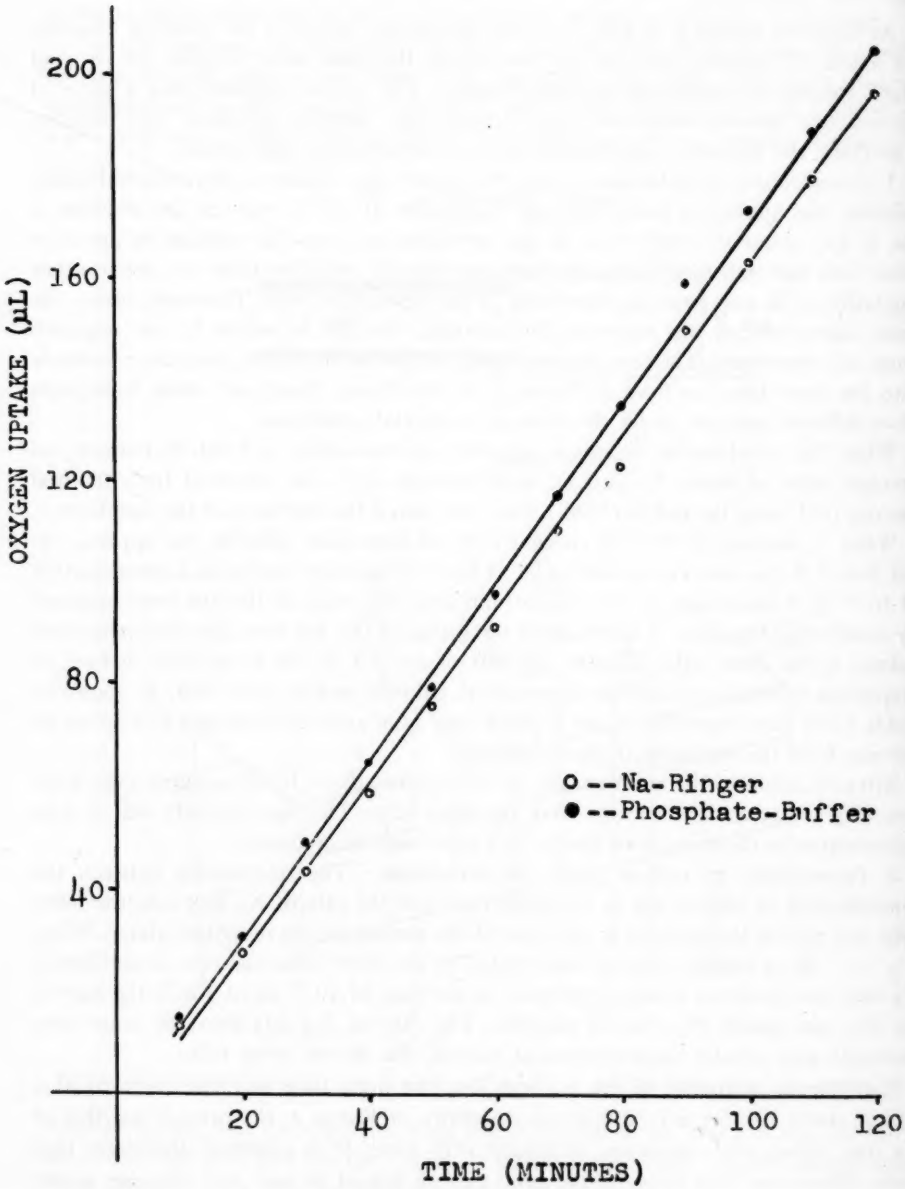

FIG. 2.—Oxygen uptake of oral mucosa

3. *Permeability of calcium across the membrane.*—The pattern of transport of calcium ions through oral mucosa was examined in order to detect some difference between the monovalent cations and the divalent cations in their permeability across the membrane. If passive diffusion was the main process by which inorganic ions were transported through the membrane, one would expect the transport of divalent cations to have a pattern similar to that of the monovalent cations.

Concentrations of 10^{-3} M and 2×10^{-3} M of $CaCl_2$ dissolved in 0.9 per cent NaCl solution were used as the experimental solutions.

The rate of spontaneous migration of calcium ions was about 5 r/ml/10 minutes. Even when $CaCl_2$ (concentrations of 10^{-3} M or 2×10^{-3} M) and 10^{-3} M $Ca(H_2PO_4)_2$

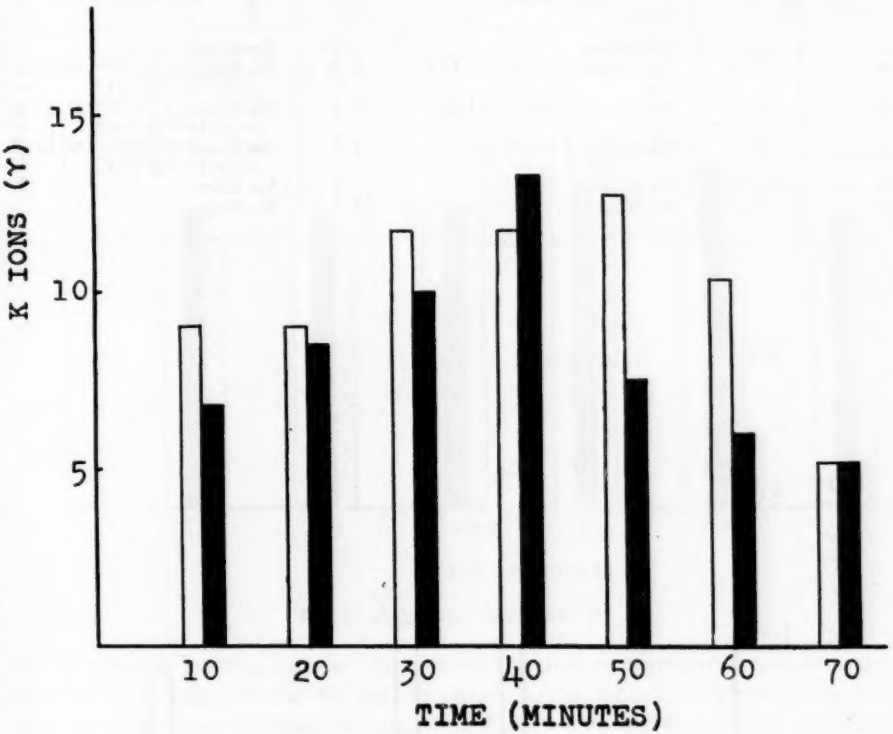

FIG. 3.—Migration rate of K ions from oral mucosa

TABLE 1

RATE OF NET FLUX OF K IONS

Time (Min.)	K Ion (γ)	Outer Medium	K Ion (γ)	Outer Medium
20.....	9.4	Na-Ringer	9.5	Na-Ringer
40.....	44.0	Na-Ringer $+10^{-3}$ M KCl	46.0	Na-Ringer $+10^{-2}$ M KCl
60.....	82.0	Na-Ringer $+10^{-3}$ M KCl	95.0	Na-Ringer $+10^{-2}$ M KCl
80.....	95.5	Na-Ringer $+10^{-3}$ M KCl	.....	.....

were placed in the outer tube, the Ca ions in the inner tube did not increase in number. A net flux of Ca ions across the membrane was not detected. Figure 5 shows this result. However, about 20–25 r/ml of Ca ions were detected from the oral mucosa itself when treated in this experiment for about 1 hour, and the volume of Ca ions contained in these treated membranes was about four to five times higher than that of a control fresh membrane.

TABLE 2

EFFECT OF DIVALENT CATION ON THE FLUX OF K IONS

Time (Min.)	K Ion (γ)	Outer Medium	K Ion (γ)	Outer Medium
10.....	10.7	Na-Ringer	6.6	Na-Ringer
20.....	10.0	Na-Ringer $+2\times10^{-3}$ M KCl	8.3	Na-Ringer $+2\times10^{-3}$ M KCl $+2\times10^{-3}$ M $CaCl_2$
30.....	12.5	Na-Ringer $+2\times10^{-3}$ M KCl	19.5	Na-Ringer $+2\times10^{-3}$ M KCl $+2\times10^{-3}$ M $CaCl_2$
40.....	22.6	Na-Ringer $+2\times10^{-3}$ M KCl	21.9	Na-Ringer $+2\times10^{-3}$ M KCl $+2\times10^{-3}$ M $CaCl_2$
50.....	31.9	Na-Ringer	37.7	Na-Ringer
60.....	13.0	Na-Ringer	23.2	Na-Ringer

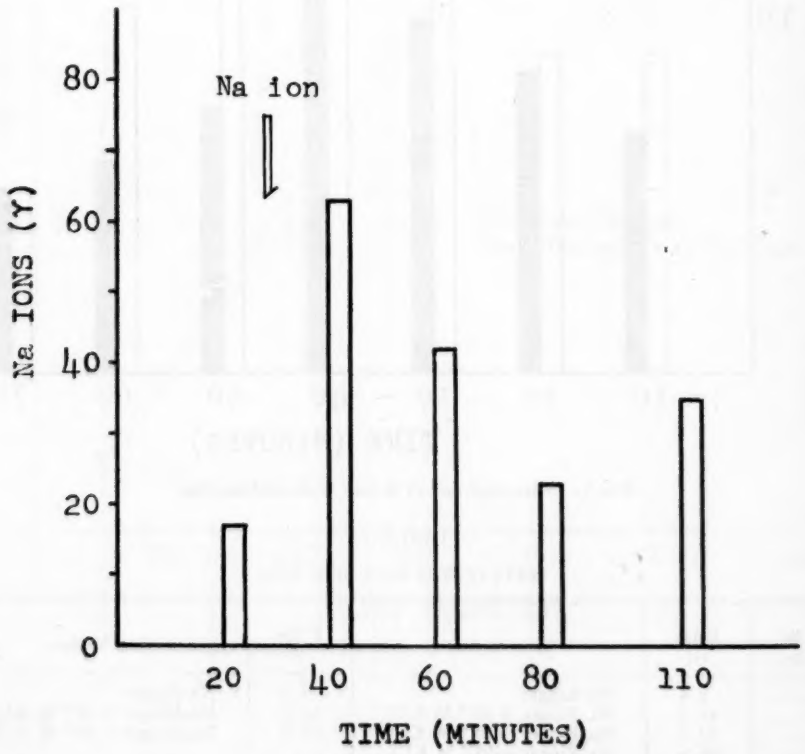

FIG. 4.—Rate of net flux of Na ions

DISCUSSION

This experiment clearly showed that the monovalent cations (Na and K) may pass into the submucosa through the oral mucosa but that the divalent cations (Ca) could not be transported through this membrane. The rate of net flux of the potassium ion across the membrane was changed in proportion to the concentration in the outer tube. It cannot be said, however, that the mechanism of this transport was only a

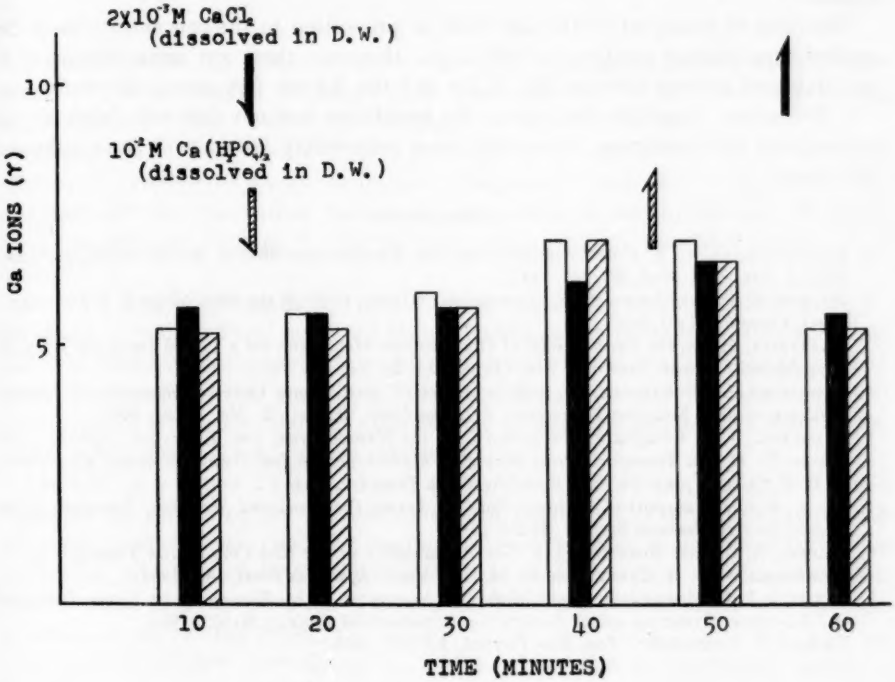

FIG. 5.—Rate of net flux of Ca ions

simple diffusion process, because the theoretical values of transferred potassium ions obtained by simple diffusion did not correspond to the experimental results. In addition, the patterns of transport of potassium and sodium ions through the membrane over a given period of time differed; therefore, some different mechanisms might exist in the transferring process.

Both of the monovalent cations migrated constantly into the inner tube, and the rate did not decrease for about 10–20 minutes after removing the load of each ion in the outer tube. This fact shows an accumulation of these ions in the membrane. For these reasons, two processes are claimed to exist in the transport of monovalent cations through the mucosa: an accumulation of ions in the membrane and their transport through the membrane.

It is not clear why the calcium ions were not transported by the oral mucosa. It was assumed that the calcium ions entered into the membrane, where they were bound with some unknown substance and, as such, were not able to permeate the membrane.

SUMMARY

The permeability of potassium, sodium, and calcium ions through the sublingual mucosa of a dog was studied. The results obtained are as follows.

1. Potassium and sodium ions were transported through the mucosa, and about 32 r/ml/20 minutes of K ions were detected when 10^{-2} M KCl was applied. The rate of transport of Na ions was about 45 r/ml/20 minutes when 5×10^{-3} M NaCl was applied.

The rates of transport of the ions were in proportion to the concentrations of the applied experimental solution in both cases. However, there are some differences in this transport process between the K ion and the Na ion flux across the membrane.

2. Transport of calcium ions across the membrane was not detected; however, Ca ions entered the membrane, where they were presumably bound with some unknown substance.

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The Inhibitory Effect of Copper Arginine on Glycolysis in Human Saliva

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Acid formation in the saliva is due to anaerobic splitting of carbohydrates by enzymes.[1, 2] Various enzyme inhibitors have been suggested in this connection, among which fluoride,[3-5] urea,[6] and soluble oxalates,[7] are known to be effective.

As long ago as 1942, Fosdick[8] drew attention to the inhibitory effect of synthetic vitamin K (2-methyl-1,4-naphthaquinone). Because vitamin K is a quinone, other quinones and their derivatives were investigated, none of which, however, displayed the same effect as vitamin K. On the other hand, various peroxides showed some activity.[9]

In 1946, Kesel, O'Donnell, Kirch, and Wach[10] reported growth inhibition of lactobacilli by ammonia. They suggested that ammonia might possibly be formed in the saliva by urea and amino acids. Ammonium diphosphate and urea produce ammonium nitrite in the saliva; this ammonium nitriate inhibits both acid formation and bacterial growth. It was also thought possible that various free amino acids occur in the saliva, which might act as enzyme inhibitors.

Fosdick[11] has tested 17 such amino acids. He evaluated the inhibition of acid formation by measuring the calcium content of human saliva after incubation with powdered enamel, sugar, and the test substance. The most active were norleucine and valine, which exerted an inhibiting effect in a concentration of 40 mg. per cent and threonine in a concentration of 20 mg. per cent. But it is doubtful whether these amino acids are present in saliva.

In 1947, Fosdick[12] investigated 31 additional substances (peroxides, quinones, guanidines, aldehydes). All these substances, with the exception of the guanidines, were found to be capable of inhibiting glycolysis. Of the aldehydes tested, glycerinaldehyde in particular proved an effective glycolysis inhibitor. This latter group of compounds is relatively non-toxic and hence most promising.

These studies show that inhibition of glycolysis is possible and that there are many compounds suitable for this purpose. However, the problem of concentration and toxicity and the fact that the substance in question should adhere to the tartar considerably reduce the number of possibilities.

In an extensive study, Fosdick[13] tested 381 compounds and determined their minimum effective concentration. Half of all the compounds investigated exerted an inhibitory effect in a concentration of 50 mg. per cent. Most antibiotics and antiseptics were active in lower concentrations, the lowest amounting to 5 mg. per cent. The two most effective substances tested were sodium-N-lauroyl sarcosinate and sodium dehydroacetate, which are already under clinical trial.

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During the enzymatic degradation of peptones, NH_3 is released, and the medium becomes alkaline, particularly if no catabolizable carbohydrates are present.[14] We have included peptone in our study, since the composition of commercially available peptones varies considerably and it is conceivable that a peptone manufactured in Europe may prove to have a more potent alkalizing effect than a peptone of different origin.

Fosdick[13] pointed out that sulfonamides display a certain degree of activity. We have supplemented his experiments by testing chlorpropamide* and N-sulfanilyl-N-*n*-butyl-carbamide,† two sulfonamides which are used in the treatment of diabetes and which influence carbohydrate metabolism. We have also tested phlorhizin, a glycoside of vegetable origin.

In addition, we have compared phenylmercuric borate,‡ a very effective antiseptic, and α-(*p*-tolyl)-dodecyl-trimethyl-ammonium-methylsulfate§ (a cationic detergent) with chlortetracycline.||

Inorganic copper salts are known to inhibit fermentation.[15] We have therefore investigated various copper compounds, such as $CuCl_2 \cdot 2H_2O$ (mol. weight 170.5) and $CuSO_4$ (159.6) and compared them with copper arginine (412).

EXPERIMENTAL METHODS

Saliva from a person subject to caries was collected after stimulation of salivary excretion and diluted to 1:10 with distilled water. The test substance was added to 2 cc. of diluted saliva in an amount expressed in the tables in terms of mg. per cent of diluted saliva. The carbohydrate was added in a concentration of 1 gm. per cent. The solutions were incubated at 37° C. The degree of enzymatic splitting of the carbohydrates was measured by potentiometric determination of the pH. The pH was measured initially and at intervals of 3, 6, 8, and 24 hours. Under normal conditions, enzymatic degradation of the carbohydrates is complete within 3 or 6 hours at the most. The 8-hour time limit is of practical significance, since human beings normally sleep for 8 hours and an ideal caries prophylactic should remain effective for this same period of time.[16] Measurements were taken after 24 hours in order to determine the final pH value.

RESULTS

Peptone, phlorhizin, chlorpropamide, and N-sulfanilyl-N-*n*-butyl-carbamide in concentrations of 10–50 mg. per cent showed little or no inhibitory effect on enzymatic splitting of glucose.

The effect of phenylmercuric borate and α-(*p*-tolyl)-dodecyl-trimethylammonium-methylsulfate compares favorably with that of chlortetracycline (Table 1). This is consistent with results reported by Fosdick.[13]

Of the three copper compounds tested in equimolar concentrations, copper arginine proved the most active (Table 2). At a concentration as low as 2.5 mg. per cent, copper arginine still inhibited glucose fermentation in the saliva (Table 3). At the same concentration it also inhibited fermentation of all other sugars and sugar deriv-

* Diabinese, Pfizer.

† Nadisan, Boehringer, Mannheim.

‡ Merfen, Zyma, Nyon.

§ Desogen, Geigy.

|| Aureomycin, Lederle.

TABLE 1

Effect of Two Antiseptics and of Chlortetracycline on Fermentation of Glucose in Saliva

	pH*				
	0 Hr.	3 Hr.	6 Hr.	8 Hr.	24 Hr.
Saliva alone	6.8	6.7	6.7	6.8	7.3
Saliva+1% glucose	6.6	6.0	5.1	4.8	3.5
Saliva+10 mg% phenylmercuric borate+1% glucose	6.6	6.5	6.3	6.3	6.2
Saliva+10 mg% α-(*p*-tolyl)-dodecyl-trimethylammonium-methylsulfate	6.6	6.9	6.9	6.8	7.1
Saliva+10 mg% α-(*p*-tolyl)-dodecyl-trimethylammonium-methylsulfate+1% glucose	6.6	6.8	6.6	6.5	5.6
Saliva+10 mg% chlortetracycline	6.4	6.6	6.6	6.5	6.6
Saliva+10 mg% chlortetracycline+1% glucose	6.4	6.6	6.6	6.6	5.8

*pH values measured 0, 3, 6, 8, and 24 hours after addition of the test substances and of glucose to a 1:10 dilution of saliva in water.

TABLE 2

Effect of Equimolar Quantities of Copper Arginine, Copper Sulfate, and Copper Chloride on Fermentation of Glucose in Saliva

	pH*				
	0 Hr.	3 Hr.	6 Hr.	8 Hr.	24 Hr.
Saliva alone	6.7	6.7	6.3	6.2	6.8
Saliva+1% glucose	6.7	6.5	5.7	5.2	4.3
Saliva+3.87 mg% $CuSO_4$	6.8	6.9	7.0	7.0	6.1
Saliva+3.87 mg% $CuSO_4$+1% glucose	6.8	6.8	6.3	5.7	4.4
Saliva+4.13 mg% $CuCl_2 \cdot 2H_2O$	6.7	6.8	6.9	7.0	6.9
Saliva+4.13 mg% $CuCl_2 \cdot 2H_2O$+1% glucose	6.7	6.8	6.8	6.9	5.9
Saliva+10 mg% copper arginine	6.8	7.0	7.1	7.1	7.3
Saliva+10 mg% copper arginine+1% glucose	6.8	7.0	7.0	7.1	7.2

*pH values measured 0, 3, 6, 8, and 24 hours after addition of the test substances and of glucose to a 1:10 dilution of saliva in water.

TABLE 3

Effect of Various Concentrations of Copper Arginine on Fermentation of Glucose in Saliva

	pH*				
	0 Hr.	3 Hr.	6 Hr.	8 Hr.	24 Hr.
Saliva alone	6.7	6.3	6.3	6.4	6.8
Saliva+1% glucose	6.7	5.8	4.7	4.5	3.8
Saliva+50 mg% copper arginine	6.9	7.1	7.2	7.2	7.2
Saliva+50 mg% copper arginine+1% glucose	6.8	7.1	7.2	7.2	7.2
Saliva+25 mg% copper arginine	6.8	7.0	7.1	7.1	7.1
Saliva+25 mg% copper arginine+1% glucose	6.8	7.0	7.1	7.1	7.1
Saliva+10 mg% copper arginine	6.8	7.0	7.1	7.1	7.1
Saliva+10 mg% copper arginine+1% glucose	6.7	7.0	7.1	7.0	7.0
Saliva+5 mg% copper arginine	6.8	6.9	6.9	6.9	7.0
Saliva+5 mg% copper arginine+1% glucose	6.7	6.8	6.9	6.9	7.0
Saliva+2.5 mg% copper arginine	6.7	6.8	6.9	6.9	6.9
Saliva+2.5 mg% copper arginine+1% glucose	6.6	6.7	6.8	6.8	6.8

*pH values measured 0, 3, 6, 8, and 24 hours after addition of copper arginine and of glucose to a 1:10 dilution of saliva in water.

atives tested, a slight rise in pH being observed 3–8 hours after the onset of the experiment (Table 4).

SUMMARY

The decrease in pH of the saliva within a given time interval was interpreted as evidence of the degree of glycolysis.

Several sulfonamides which influence carbohydrate metabolism, such as N-sulfanilyl-N-*n*-butyl-carbamide and chlorpropamide, showed no inhibitory effect on fermentation. The same applied to phlorhizin and peptone.

TABLE 4

EFFECT OF COPPER ARGININE ON FERMENTATION OF VARIOUS SUGARS AND SUGAR DERIVATIVES IN SALIVA

	pH*				
	0 Hr.	3 Hr.	6 Hr.	8 Hr.	24 Hr.
Saliva alone	6.4	6.5	6.1	5.9	5.8
Saliva+2.5 mg% copper arginine	6.4	6.5	6.6	6.6	6.7
Saliva+1% glucose	6.5	6.5	5.4	4.6	3.8
Saliva+2.5 mg% copper arginine+1% glucose	6.5	6.7	6.7	6.7	6.3
Saliva+1% fructose	6.5	6.4	5.7	4.9	3.8
Saliva+2.5 mg% copper arginine+1% fructose	6.5	6.6	6.6	6.6	6.2
Saliva+1% maltose	6.4	6.3	5.0	4.4	3.9
Saliva+2.5 mg% copper arginine+1% maltose	6.4	6.4	6.5	6.5	5.9
Saliva+1% lactose	6.5	6.4	5.5	4.7	4.1
Saliva+2.5 mg% copper arginine+1% lactose	6.5	6.5	6.5	6.6	6.4
Saliva+1% sucrose	6.4	6.3	5.9	5.1	4.0
Saliva+2.5 mg% copper arginine+1% sucrose	6.4	6.5	6.6	6.6	6.6
Saliva+1% salicin	6.4	6.4	5.8	5.2	4.8
Saliva+2.5 mg% copper arginine+1% salicin	6.4	6.5	6.5	6.6	6.5
Saliva+1% raffinose	6.3	6.4	5.6	4.7	4.5
Saliva+2.5 mg% copper arginine+1% raffinose	6.3	6.5	6.5	6.6	6.4
Saliva+1% glycerin	6.3	6.4	5.9	5.8	5.8
Saliva+2.5 mg% copper arginine+1% glycerin	6.3	6.5	6.6	6.6	6.6
Saliva+1% sorbite	6.4	6.4	6.3	6.1	5.3
Saliva+2.5 mg% copper arginine+1% sorbite	6.4	6.6	6.7	6.7	6.8
Saliva+1% arabinose	6.4	6.4	6.0	5.7	4.6
Saliva+2.5 mg% copper arginine+1% arabinose	6.4	6.5	6.7	6.7	6.4
Saliva+1% mannite	6.4	6.4	6.3	6.0	5.3
Saliva+2.5 mg% copper arginine+1% mannite	6.4	6.5	6.7	6.7	6.8
Saliva+1% amylum	6.5	6.5	5.9	5.0	4.0
Saliva+2.5 mg% copper arginine+1% amylum	6.5	6.7	6.8	6.8	6.5

* pH values measured 0, 3, 6, 8, and 24 hours after addition of copper arginine and of sugar or sugar derivatives to a 1:10 solution of saliva in water.

Phenylmercuric borate, an effective antiseptic, and α-(*p*-tolyl)-dodecyl-trimethyl-ammonium-methylsulfate, a cationic detergent, were not superior to chlortetracycline with regard to inhibition of fermentation.

Copper arginine appeared to be particularly effective as a fermentation inhibitor. It was superior to $CuCl_2 \cdot 2H_2O$ and $CuSO_4$ when used in equimolar quantities.

Copper arginine at a concentration of 2.5 mg. per cent inhibited fermentation of all sugars and sugar derivatives tested.

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Dental Caries and pH on the Tooth Surfaces of Albino Rats Fed a Cariogenic Diet Containing Sodium-N-Lauroyl Sarcosinate

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Several years ago the idea was proposed by Fosdick[1] that an enzyme inhibitor which would effectively block the formation of acid from carbohydrates should serve as a prophylactic agent against dental caries. This reasoning was based on the hypothesis that the initial lesion of dental caries is produced by acid formed on a tooth surface by the enzymatic degradation of carbohydrates. It was thought that brushing the teeth with a tooth paste in which an enzyme inhibitor was incorporated should serve as an effective means for the prevention or control of dental caries. Fosdick's criteria for an inhibitor satisfactory for this purpose are as follows: it should be (1) effective in the prevention of acid formation from sugar; (2) capable of attaching itself to the plaque, thereby remaining active for a period of several hours; (3) relatively nontoxic and colorless; (4) not unpleasant to the taste; and (5) readily available.

A large screening program indicated that sodium-N-lauroyl sarcosinate (SLS)* met these specifications. It resisted removal from the plaque, and over a period of several hours decreased the rate of acid formation in the plaque that had been removed from the teeth after the mouth had been rinsed for 30 seconds with a 50 per cent sucrose solution.

Subsequently, a two-year clinical study was conducted from which it was concluded that brushing the teeth with a dentifrice containing SLS resulted in a reduction in the annual increment of dental caries.[2, 3] In a later study by Hayden and Glass,[4] the addition of SLS to tooth paste appeared to offer some protection against caries in the teeth of school children.

Animal experiments have given contradictory results on the effectiveness of SLS in the reduction of dental caries. Volker, Apperson, and King[5] found that this compound, when incorporated in the diet, had but a very slight protective action against caries in the hamster, whereas, in the experiments of Zipkin and McClure,[6] the addition of 0.5 per cent SLS to a cariogenic diet resulted in a significant reduction in the severity of both sulcal and smooth-surface lesions. Keyes and White,[7] on the other hand, obtained no reduction in caries in the sulcal areas but less smooth-surface caries in Sprague-Dawley rats fed a fine-particle cariogenic diet and given drinking water containing 0.5 per cent SLS. These authors refer to personal communications with other

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* The sodium-N-lauroyl sarcosinate was kindly supplied us in generous amounts through the courtesy of Dr. W. J. King, Colgate-Palmolive Co.

investigators who found that the addition of SLS to a cariogenic diet produced little or no effect on caries activity in albino rats and hamsters.

With regard to the prevention of acid formation in the plaque, some doubt has been raised as to the effectiveness of SLS. Forscher and Hess[8] obtained the same pattern of acid formation in the plaque of subjects using a dentifrice containing 2 per cent SLS as in that of control subjects. Brudevold, Little, and Rowley[9] failed to find any prolonged effect of SLS on acid production in the plaque from interdental spaces. Furthermore, the acid-reducing effect which they found on the labial plaque was considerably less noticeable than that which Fosdick reported on the plaque from interdental spaces. They concluded that either positive or negative results may be obtained, depending on the type of plaque selected for pH measurements. Hartles[10] found that SLS inhibited to some extent glycolysis in saliva sediments but that it was much less effective than sodium-lauroyl sulfate.

In view of the divergent results reported in the literature on the reduction in dental caries in animal experiments and on the inhibition of acid production by SLS, the present experiments were undertaken to determine whether the addition of SLS to a high-sucrose cariogenic diet fed to albino rats would affect (1) the pH on the surface of the teeth and (2) the cariogenicity of the diet.

Three experiments were conducted—one on sialoadenectomized and two on intact animals. One of these latter experiments was done on a caries-resistant and the other on a caries-susceptible strain.

EXPERIMENT I

Methods.—In the first experiment twenty-five pairs of littermates of the Emory-Wistar strain were sialoadenectomized at weaning by our usual procedure. From this time on, one animal of each pair was fed a finely ground high-sucrose cariogenic diet and its littermate the same diet to which was added 0.5 gm. Na-N-lauroyl sarcosinate per 100-gm. diet. Each animal was kept in an individual cage and allowed to eat ad libitum. As caries usually occurs earlier and more extensively in the first molar of these animals, this tooth was selected as the locus for obtaining the pH readings. The antimony electrode was used for all pH determinations. Since caries is initiated at the bottom of the fissure and since it is impossible to construct an electrode small enough to pass into the fissure, the latter was enlarged by drilling with a bur having a slightly larger diameter than the electrode. We were thereby enabled to bring the tip of the electrode in contact with the bottom of the fissure. The procedures of enlarging the fissure and the construction of the antimony electrode are described elsewhere.[11]

The food cups were removed from the cages in the evening, and the pH readings were taken in mid-morning the next day. After the pH at the bottom of the second fissure of the right and left first molars had been determined, the food cups were placed in the cages and the animals allowed to eat for 10 minutes. The food cups were then removed, and the pH was determined immediately and then again 10 and 20 minutes later. The pH readings were taken at intervals of approximately 6–8 days throughout the experimental period of 110 days.

In the course of the experiment a total of 350 readings was taken before eating and the same number at each of the various time intervals after eating. At the con-

clusion of the experiment the animals were sacrificed and the teeth scored for caries by our customary procedure.[12]

Results.—The average pH values are presented in Table 1 and the caries scores in Table 2. There was a significant reduction in the amount of caries in the animals fed the diet containing SLS, whereas there was no difference in the pH on the teeth of the animals fed the test and the control diets.

TABLE 1

pH ON SURFACES OF TEETH OF SIALOADENECTOMIZED EMORY-WISTAR RATS FED CARIOGENIC DIET CONTAINING 0.5 PER CENT SODIUM-N-LAUROYL SARCOSINATE

Diet	No. of Animals	Days on Diet	pH*			
			Before Eating	Immediately after Eating	10 Min. Later	20 Min. Later
Control (cariogenic).....	25	110	7.9	7.2	7.2	7.2
Control plus SLS.....	25	110	7.8	7.3	7.1	7.3

* Each pH value is an average of 350 readings.

TABLE 2

DENTAL CARIES OF SIALOADENECTOMIZED EMORY-WISTAR RATS FED CARIOGENIC DIET CONTAINING 0.5 PER CENT SODIUM-N-LAUROYL SARCOSINATE

Diet	No. of Animals	Days on Diet	Carious Lesions*	S.E.M.	C.R.†	Caries* Score	S.E.M.	C.R.†
Control (cariogenic)....	20	110	23	±0.61	4.5	45	±4.3	3.9
Control plus SLS.....	20	110	19	±0.67		26	±2.3	

* Each value is an average per rat.

† The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. When the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; 2.0–2.9, the difference is of borderline significance; 3.0 or more, the difference is highly significant (J. M. Dunning, *J. D. Res.*, **29**:541, 1950).

EXPERIMENT II

Methods.—The second experiment was conducted in a manner similar to the preceding one on the same strain of rats except that the lesser number of animals were not sialoadenectomized. As these animals are resistant to caries when the salivary glands are intact, they were fed the cariogenic diet for 330 days.

Results.—The results of this experiment are presented in Tables 3 and 4. There was an insignificant amount of caries in these animals, and there was no difference between the tests and the controls. As in the case of the sialoadenectomized animals, there was no difference in the pH on the teeth of the test and control animals. It will be noted that the pH readings on the teeth were somewhat higher than those on the sialoadenectomized animals. This has been the usual finding in our laboratories.[11]

EXPERIMENT III

Methods.—In view of the very small amount of caries in any of these animals, it was decided to run another experiment on intact animals of a more susceptible strain. These animals were obtained from a colony in our laboratories developed from several pairs that had been obtained some years ago from the Naval Institute of Medical Research. At the conclusion of a 120-day feeding period on the same cariogenic diet that was used in the preceding experiments, the animals were sacrificed and the teeth scored for dental caries. In this experiment a number of pH readings were taken, but, as they were essentially the same as those given in Table 4, this time-consuming procedure was discontinued.

Results.—The results of the caries evaluation are presented in Table 5. In this ex-

TABLE 3

pH ON SURFACES OF TEETH OF INTACT EMORY-WISTAR RATS (CARIES-RESISTANT STRAIN) FED CARIOGENIC DIET CONTAINING 0.5 PER CENT SODIUM-N-LAUROYL SARCOSINATE

Diet	No. of Animals	No. of Days on Experiment	pH*			
			Before Eating	Immediately after Eating	10 Min. Later	20 Min. Later
Control (cariogenic).....	10	330	8.2	7.3	7.6	8.0
Control plus SLS.....	10	330	8.2	7.3	7.5	7.8

* Each pH value is an average of 230 readings taken at intervals of approximately 14 days.

TABLE 4

DENTAL CARIES OF INTACT EMORY-WISTAR RATS (RESISTANT STRAIN) FED CARIOGENIC DIET CONTAINING 0.5 PER CENT SODIUM-N-LAUROYL SARCOSINATE

Diet	No. of Animals	Days on Diet	Carious Lesions*	S.E.M.	C.R.	Caries* Score	S.E.M.	C.R.
Control (cariogenic).....	10	330	10	±1.6	0.4	12	±3.0	0.3
Control plus SLS.....	10	330	9	±1.7		13	±3.1	

* Each value is an average per rat.

TABLE 5

DENTAL CARIES OF INTACT NIMR RATS (CARIES-SUSCEPTIBLE STRAIN) FED CARIOGENIC DIET CONTAINING 0.5 PER CENT SODIUM-N-LAUROYL SARCOSINATE

Diet	No. of Animals	Days on Diet	Carious Lesions*	S.E.M.	C.R.	Caries* Score	S.E.M.	C.R.
Control (cariogenic).....	20	120	20	±0.4	6.3	52	±1.7	7.3
Control plus SLS.....	20	120	16	±0.5		32	±2.2	

* Each value is an average per rat.

periment on intact animals of a caries-susceptible strain as in the experiment on sialoadenectomized rats of a caries-resistant strain, the addition of SLS to the diet resulted in a significantly lower incidence of caries and a smaller caries score.

DISCUSSION

As there was no difference in the pH on the teeth of the test and control animals in any of the experiments, it may be concluded that the cariostatic action of the SLS cannot be attributed to an influence of the compound on the pH of the tooth surface. One must therefore seek a different explanation of its mode of action than the one that has been proposed by Fosdick.

It has been shown by Zipkin and McClure[9] that the ability of this compound to inhibit dental caries is unrelated to its detergent properties. Shklair, Hoerman, and Englander[13] have shown that SLS inhibits the growth of lactobacilli. Rosenthal, Marson, and Abriss[14] also found that this compound has a bacteriostatic action against *Lactobacillus acidophilus*. We have conducted several tests to determine the effect of SLS on bacterial growth in the concentration used in the experiments reported in this paper. Swabs taken from the surface of the molar teeth in the right quadrant of 6 human subjects were streaked on Rogosa LBS agar in Petri dishes, and swabs from the opposite quadrant were streaked on the same medium containing 0.5 per cent SLS. After incubation, there were a few colonies (presumably lactobacilli) on the LBS agar but none on the SLS-containing medium.

In another experiment a rat that had been fed our cariogenic diet was sacrificed and the teeth removed and ground under sterile conditions according to the procedure described by Wakeman, Smith, Sarles, Phillips, and Elvehjem.[15] The ground material was thoroughly mixed with sterile 0.9 per cent NaCl solution. On each of 6 plates consisting of LBS agar, there was placed 0.5 cc. of the suspension and the same amount on 6 plates containing the LBS agar plus 0.5 per cent SLS. Upon incubation, bacterial colonies were present on the LBS plates but not on those to which SLS had been added.

As it has not been established that lactobacilli are the organisms directly responsible for dental caries, another experiment was conducted. Two sets of Petri dishes were prepared, one containing nutrient agar and the other nutrient agar plus 0.5 per cent SLS. A swab was taken from the buccal surface of the lower left first molar of 5 rats and streaked on the nutrient agar plates. Immediately thereafter another swab was taken on the lower right first molar and streaked on the agar containing SLS. The plates were then incubated for 72 hours. On all the plates of nutrient agar alone, there was a prolific growth of bacteria, whereas the agar-SLS plates remained absolutely clear. The experiment was then repeated on the subjects, reversing the procedure. The swabs from the left molar were streaked on the plates containing the SLS and those from the right molars on the agar without SLS. The results were the same as in the preceding experiment.

In another experiment, the pH of the agar-SLS was adjusted to that of the agar itself by adding 0.1 ml. 3 N HCl to 500 ml. agar. Several of the agar-SLS plates were streaked with swabs from the first molars of 3 of the rats used in the preceding two experiments and incubated for 72 hours. As in the other experiments, the plates remained free of bacterial growth. This ruled out the possibility that the failure of bacteria to grow on the agar-SLS plates may have been due to any change in the pH

of the medium that may have been brought about by the addition of the sarcosine. These observations suggest that the anticariogenic property of SLS manifested in our experiments was, in all probability, due to a bacteriostatic or bacteriocidal action of the compound.

SUMMARY

The effect of sodium-N-lauroyl sarcosinate added to a cariogenic diet on dental caries and on the pH on the surface of the teeth has been studied in sialoadenectomized and intact rats.

The addition of this compound to the diet had a somewhat inhibitory effect on dental caries but did not affect the pH on the tooth surface.

No bacterial growth was obtained on agar plates containing 0.5 per cent SLS when streaked with swabs from human teeth, whereas prolific growth occurred on pure agar.

It is concluded that the cariostatic action of SLS observed in these experiments cannot be attributed to an influence on the pH on the tooth surface but was probably due to its bacteriostatic or bacteriocidal properties.

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An Electromyographic Analysis of the Temporalis Muscles and Certain Facial Muscles in Thumb- and Finger-sucking Patients

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Every dentist is frequently confronted with the problem of digital sucking in children. Yet any information he can give questioning parents relies largely on opinions, observations, or argumentation, because very little scientific evidence is found in the literature.

In 1958, Cook[1] analyzed intraoral pressures involved during thumb- or finger-sucking in a selected sample of 25 children. He found that there was a relationship between the type of pressure developed and the malocclusion observed.

After such findings, one is naturally tempted to define more precisely the part played by the digital pressure and that played by the neuromuscular complex. Is the sucking habit a source of alteration in facial muscle function? If so, what muscles are mostly involved? The purpose of this study is to examine electromyographically the involvement of selected fifth and seventh cranial nerve muscles in a group of thumb- and finger-suckers.

EXPERIMENTAL METHODS

The subjects in the present study included 24 of the 25 subjects investigated by Cook.[1] They all had, or had had in the previous six months, an active thumb- or finger-sucking habit. The age range was five years and ten months to ten years and eleven months, with a mean of eight years and six months (Table 1).

An eight-channel electroencephalograph* modified for electromyography was used (Fig. 1). Each subject was placed in a Faraday cage to minimize electrical interference. The individual calibration of amplification for the pen-writers was 6 mm/50 microvolts for the temporalis and buccinator muscles and 4 mm/50 microvolts for the orbicularis oris and mentalis muscles. Only the "all-channel amplification control" was used for varying the gain after the recording was started. This control alters the amplification simultaneously in all recording channels. It was necessary to use it to decrease the amplitude for all subjects during biting and for some of them during sucking. Compensation for such changes in gain was made at the time of the analysis. The linear response had a frequency range between 5 and 70 cycles/sec. Muscular

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* Grass model IV-A.

responses below or above this range are not accurately recordable by the ink writer. Only the records at paper speed of 60 mm/sec were analyzed in this study.

Electrodes.—Two types of electrodes were used: (1) monopolar surface electrodes of approximately 3 mm. in diameter, which were held in place by means of bentonite paste and cotton pressed over the electrodes. The selected area was cleaned with alcohol and rubbed briskly with the bentonite paste, in order to produce erythema and reduce the surface resistance; (2) monopolar needle electrodes, consisting of a silver disk of approximately 8 mm. in diameter, in the center of which a 30-gauge needle was fixed by means of insulating acrylic. The needle itself was insulated to the extreme tip and bent at 90° in order to avoid a long lever arm (Fig. 2). Electrode placement was as follows:

Temporalis muscle—the middle fibers of this muscle were studied, bilaterally, and the surface electrodes were positioned as described by Moyers.[2]

TABLE 1

INFORMATION PERTAINING TO THE SAMPLE

Subject	Age	Anteroposterior			Transverse Cross-Bite	Vertical		Swallowing Pattern*
		Molar Class Angle	"B" pt. to "A"	Over-Jet		Overbite	Open Bite	
Group I:								
J.B.	83	I	0.6	8.0	.....	4.0	X	S
J.V.	104	II	−7.9†	11.0	.....	−1.0	.....	V
D.A.	111	II	0.0	6.4	X	−0.8	.....	S
J.H.	113	II	−0.2	6.5	.....	−1.1	.....	T
L.L.	125	II	1.0	9.5	X	−3.0	.....	T
Group II:								
R.G.	70	I	1.9	2.0	.....	−1.4	.....	S
D.L.	74	II	−3.9†	7.5	.....	0.0	X	V
C.E.	74	I	0.0	10.0	.....	9.0	X	S
G.F.	97	II	0.0	8.3	X	0.0	X	V
E.A.	98	I	−0.5	8.0	.....	6.8	X	T
J.R.	101	I	−0.5	6.8	.....	−2.0	.....	T
J.F.	106	II	0.0	5.6	X	2.4	X	S
N.A.	106	II	−1.1	10.0	.....	0.0	X	S
L.B.	110	I	−4.5†	5.9	X	0.0	X	V
F.H.	111	II	−3.9†	5.6	.....	−3.0	.....	V
G.A.	116	I	−3.0†	7.9	.....	0.0	X	S
C.P.	116	II	−2.5†	5.9	.....	−4.1	.....	V
N.H.	117	II	0.8	6.2	.....	−1.0	.....	V
R.W.	118	I	5.0	3.5	X	0.0	X	S
P.J.	126	II	2.8	3.6	X	0.0	X	V
E.T.	131	II	−3.1†	7.2	.....	−2.9	.....	V
Group III:								
J.G.	75	I	0.9	3.3	.....	−2.1	.....	T
E.B.	79	I	−1.6	2.3	X	1.8	X	V
T.E.	126	I	3.3	5.0	.....	−4.3	.....	S
Mean	.....	.....	−0.68	6.3	.....	−0.15	.....	.....
	.....	.....	.....	7.28†	.....	.....	.....	.....
S.D.	.....	.....	2.81	2.43	.....	3.14	.....	.....
Range	.....	.....	−7.9 to 5.0	2.0	.....	−4.3 to 9.0	.....	.....

* The classification into three groups is based on evidence introduced later. V = visceral; T = transitional; and S = somatic swallowers.

† Skeletal Class II.

Mentalis muscle—needle electrodes did not show any advantage over the surface electrodes; therefore, the latter were used and placed bilaterally, approximately 8 mm. apart, over the incisive fossa.

Orbicularis oris—the upper lip was divided into six equal segments, and three surface electrodes were positioned on each side. It was possible to show that any one of the three unilateral divisions was as good as the others but that certain movements would produce asymmetry of contraction when comparing right to left. It was then decided to use the middle division, bilaterally, to study the orbicularis oris contractions (Fig. 3).

Buccinator muscle—a line joining the tragus of the ear to the commissure of the lip was explored with a probing needle electrode. A region was found, approximately half-

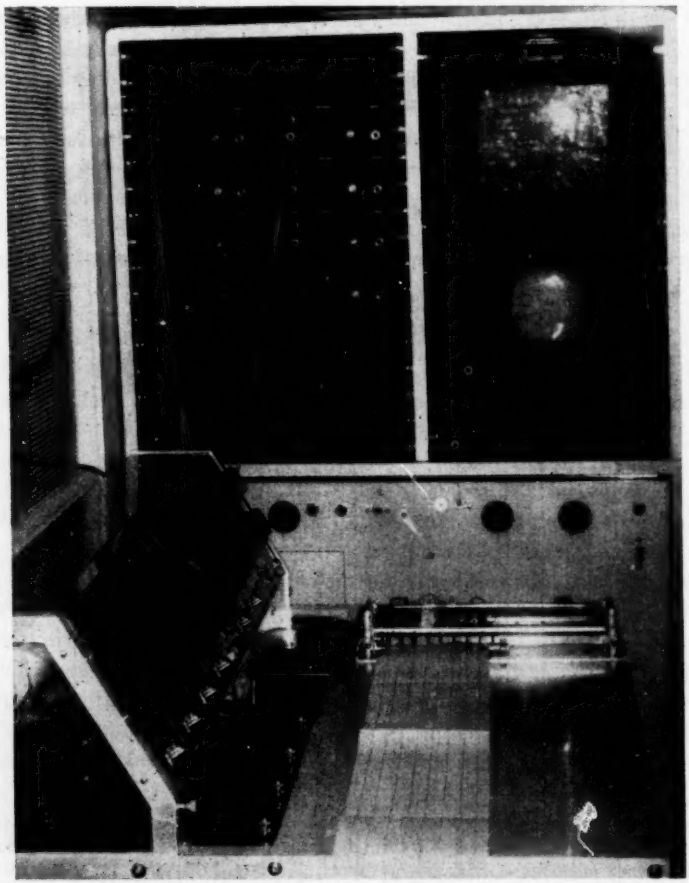

FIG. 1.—An eight-channel Grass Electroencephalograph, model IV-A, modified for electromyography. Note also the single-channel and eight-channel oscilloscopes which were used occasionally for monitoring and the window of the Faraday cage through which commands were given and the subjects were observed.

way between the commissure of the lip and the anterior border of the masseter (determined by palpation), where the overlapping of fields, as described by Hickey,[3, 4] was minimal. The subject was asked to bite as hard as he could, and no contraction was registered by the needle. This was done to avoid interpreting a masseteric contraction as a buccinator one. However, it was realized that the proximity of the zygomatic, caninus, and levator labii muscles—all seventh cranial nerve muscles—was another source of overlapping fields. Therefore, this region and group of muscles will be referred to as the "buccal wall."

The ear lobe was used as reference for the temporalis muscle, while the nose was used for the mentalis, orbicularis oris, and buccal-wall muscles. At the start and end of each session, the instrument was calibrated, and an electrode resistance test was taken. For all records, the resistance was under 5,000 ohms. The room was also kept as quiet as possible to help relaxation of the patient.

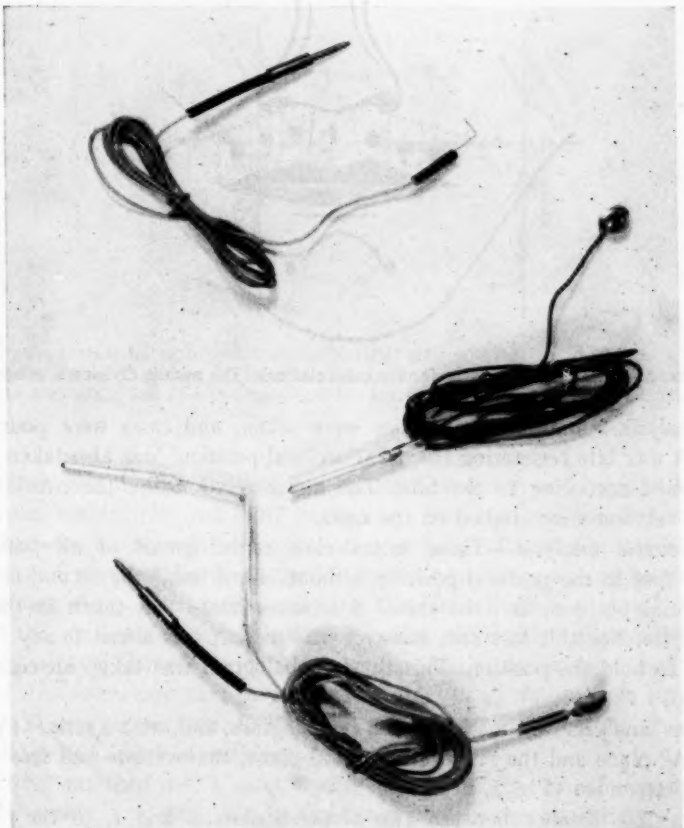

FIG. 2.—Needle and surface electrodes; needle was made by Meditron Co. and was transformed into a monopolar needle electrode as seen in the bottom. The needle was insulated by the Meditron Co. to the extreme tip.

Movements.—Each subject was asked to do the following exercises in the order given:

1. Relax
2. Swallow
3. Tap
4. Bite hard
5. Relax
6. Swallow
7. Relax
8. Suck
9. Relax
10. Swallow
11. Tap
12. Bite hard
13. Relax
14. Swallow

Numerous repetitions of swallowing, tapping, and biting were asked. In exercise No. 8, the subject was told to suck his thumb or finger naturally, as long as it pleased him. Exercise No. 9 was done as soon as the subject removed his thumb from his mouth. Finally, at the completion of exercise No. 14, a three-minute rest period was given and exercises 8–14 were repeated.

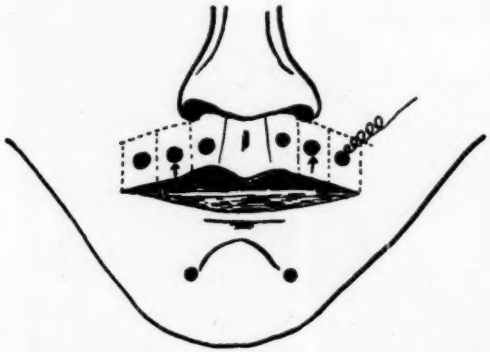

FIG. 3.—Electrode placement for the orbicular oris. The middle division was used

Cast analysis.—Alginate impressions were taken, and casts were poured for all subjects. A wax bite registering the usual occlusal position[5] was also taken, and casts were trimmed according to the bite. The molar relationship, the overjet, and the transverse relation were studied on the casts.

Cephalometric analysis.—Three lateral-view cephalograms of all patients were taken, the first in the postural position without instruction,[6] the second one was the usual occlusal position, and the third—5 minutes later—was taken in the postural position again. For this last one, however, the patient was asked to say "M" three times and to hold the position. This third cephalogram was taken on eight subjects only.

A Jenkins' analysis[7] was made on each cephalogram, and, with a series of perpendiculars to "A" plane and the "natural" occlusal plane, the overbite and free-way space were also determined (Fig. 5, *A* and *B*).

Overbite—the distance between two perpendiculars, *i* and *I*, to the "A" plane, tangent to the incisal edges of the most anterior lower and upper central incisors. A zero value was given to the case in which both incisal edges fell on the same plane; all positive values indicate an open bite, while a negative one indicates coverage of the lower incisors by the uppers.

Free-way space—the distance between the perpendiculars *i* and *i'*, *i'* being tangent to the edge of the most anterior lower central incisor, the mandible being in postural position. The cephalograms were superposed on the nasion and the upper central incisor.

To determine the anterior or posterior trajectory of the mandible between the occlusal position and the postural position, a line tangent to the most anterior lower incisor and perpendicular to the "natural" occlusal plane was constructed. This line was traced for both the occlusal position and the postural position (Fig. 4, *b*).

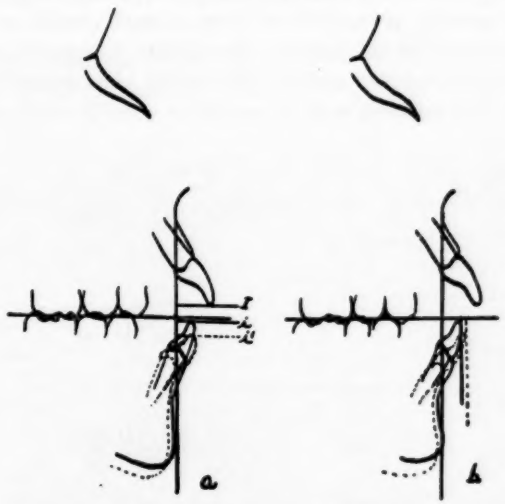

FIG. 4.—Tracings made on each cephalogram. Natural occlusal plane to "A" plane is the basis of Jenkins' analysis. Figure 4, *a*, shows the tracings made for the determination of the overbite, *I* to *i*, and of the free-way space, *i* to *i'*; *b* indicates how the anteroposterior evaluation was made.

RESULTS

In this study the primary concern was the sucking action and some of its possible neuromuscular effects. The first thing to consider, therefore, was the similarity or dissimilarity between the records before and after sucking. Muscular behavior in all subjects before and after sucking had a striking similarity; there was no basic difference in action potentials recorded before and after the demonstration of their habit. Superposition of the swallowing patterns recorded before sucking and after each one of the two sucking periods showed very little variation. Variations in the amplitude could be detected, but in no case were they consistent, and in all the subjects they appeared before as well as after sucking. It appears that neuromuscular activity is not altered for the short period following sucking.

Each action was then individually studied as to (1) what muscle was taking part in the given action; (2) what muscle had the greatest action potential; (3) what the timing or synchronization of the muscles was; and (4) what the over-all amplitude of the recordings was.

General findings on each action can be summed up as follows:

Postural position of the mandible.—Electromyography has shown that, unless the

position is conditioned, the muscles are likely to be in a state of postural contraction.[8] The postural potential being minimal, the pen-writers give a relatively straight line. Such recordings were found on 15 subjects, but 9 subjects showed mentalis contraction during the postural position, while all other muscles showed the typical minimal activity (Fig. 5). An attempt to eliminate such contractions was made during the recording session by asking the subject showing such activity to assume different mandibular positions. This was unsuccessful.

Swallowing.—This action was studied two ways: (1) the muscle showing the greatest amplitude during swallowing and (2) the type of swallowing.

According to the activity of the fifth or of the seventh cranial nerve muscles during swallowing, Tulley[9] classified his subjects, respectively, as somatic or visceral swallowers (Fig. 6). The present selected sample followed the same general pattern as Tulley's predental students. Ten subjects were found to be visceral swallowers, 8 were purely

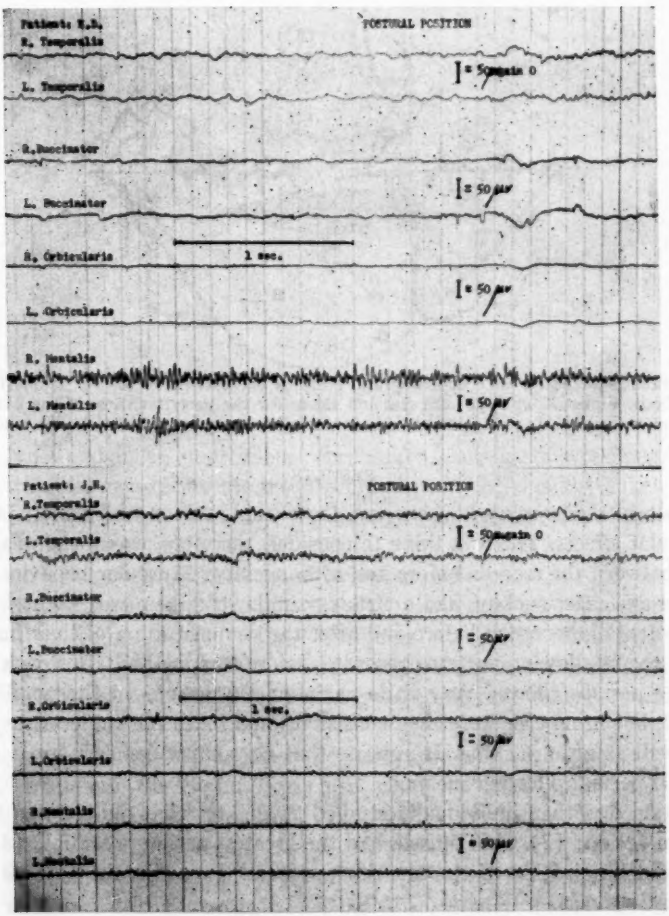

FIG. 5.—Electromyograms of two subjects in postural position. Note in *A* the mentalis muscle as compared with normal postural position shown in *B*. The gain is identical in both records.

somatic, while the remaining 6 were neither one nor the other exclusively. Four different patterns were found in this last group: (1) delayed contractions of the temporalis as compared to the other muscles; (2) minimal contractions of the temporalis, smaller in amplitude than the contractions of the seventh cranial nerve muscles; (3) visceral and somatic swallowing patterns equally mixed during the entire recording session; and (4) a mixture of the above three. Henceforth this latter group will be called the "transition" group in reference to Rix's "transition" period.[10] He labeled the period of life when the child changes from the infantile tooth-apart swallow to a so-called adult teeth-in-contact swallow as the transition period.

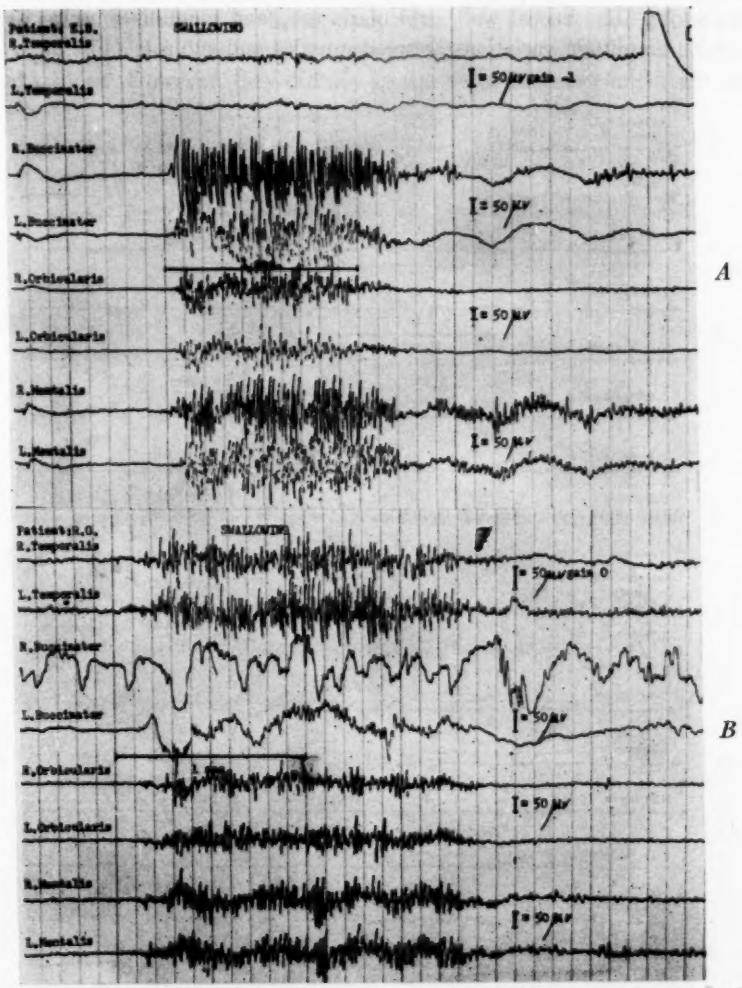

FIG. 6.—Electromyograms of two subjects during swallowing. *A* is a visceral swallower, and *B* is a somatic swallower. Note that the gain was decreased in *A* because of the great amplitude of contraction of the mentalis. The minimal contraction of the temporalis muscles in *A* is typical, and it is apparent that the seventh cranial nerve muscles are dominant during the swallowing act.

Tapping and biting.—Both actions are primarily mediated by the fifth cranial nerve muscles. However, 19 subjects have recorded mentalis contractions during each one of these movements, while only 5 have shown contractions of the temporalis muscles alone. The 9 subjects who showed contraction of the mentalis muscles during the postural position gave a long-sustained contraction of the mentalis during tapping and biting (Fig. 7). The other 10 have produced rhythmical contractions of the mentalis synchronized with the temporalis muscles (Fig. 8). The lip showed minimal action potential during tapping and biting, and buccal-wall contractions classified as negligible were seen on the electromyograms of 4 subjects only.

Sucking.—As one would expect, the greatest display of action potential was recorded during sucking. The buccal wall, orbicularis oris, and mentalis muscles were always active, with amplitude variations between muscles and subjects (Fig. 9). No subject

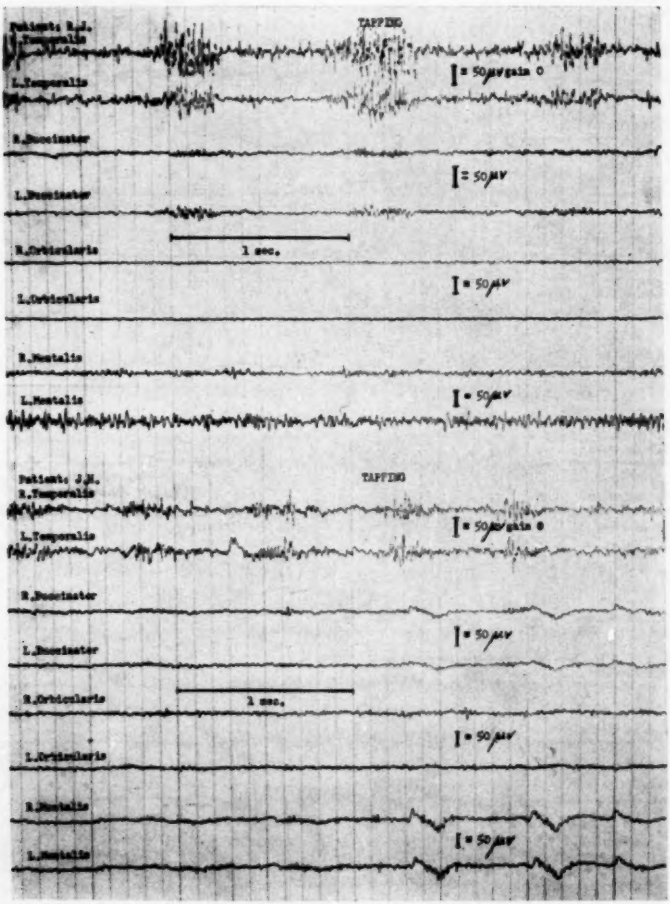

FIG. 7.—Electromyograms of two subjects during tapping. A long-sustained contraction of the mentalis muscles is seen in *A*, while *B* is the picture of a subject showing contraction of the temporalis muscle alone during tapping. The gain is the same in both cases.

showed any significant contraction of the temporalis muscles during sucking. These muscles showed the same behavior in sucking as they showed in postural position (Figs. 9 and 10).

It also appeared that sucking could be classified under rhythmical or sustained patterns (Fig. 10). Such a classification failed to show a correlation with any of the collected data.

The average time of sucking was 66.7 seconds for one period; the range was 14–150 seconds. Such a wide range for a sample of 24 gives the time element a non-statistically significant value.

The contractions of the buccal wall did not, in any instance, assume the importance expected. Though never present in tapping, biting, and the postural position, the contractions of the buccal wall appeared constantly in measurable amplitude in swallowing and sucking. However, they did not appear to be the most important contrac-

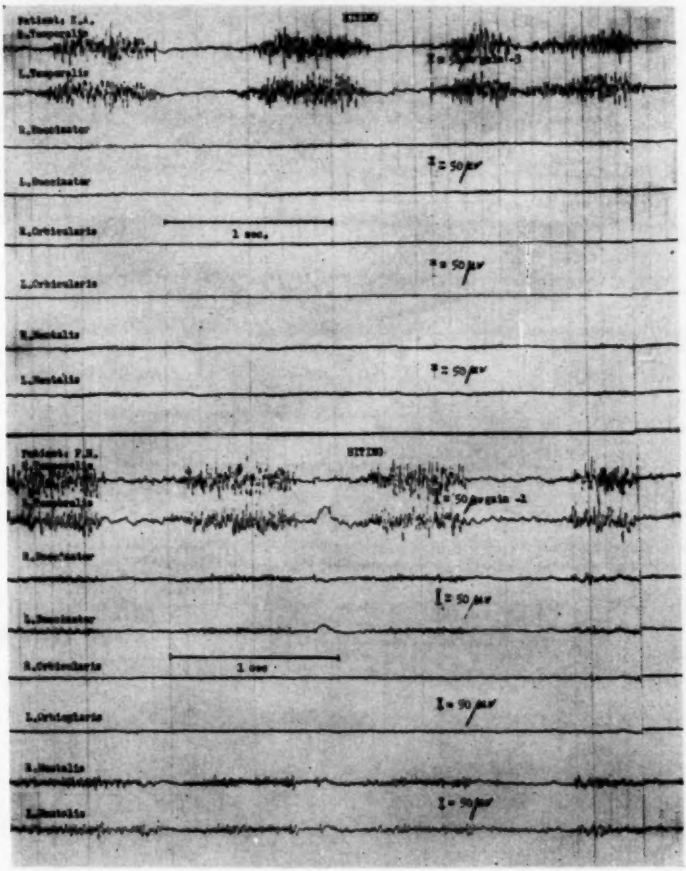

FIG. 8.—Electromyograms of two subjects during biting. *A* shows contractions of the temporalis muscles alone, while *B* shows rhythmical contractions of the mentalis muscles along with the temporalis muscles. Gain in record *A* was only two-thirds of record *B*, but the relative sensitivity between each muscle is the same.

tions in swallowing and sucking. The persistent strength of the mentalis muscles presented a surprising contrast to the weakness of the muscles of the buccal wall.

After tabulating the different actions according to the muscle activity, it was found that the general neuromuscular behavior of 21 of the 24 subjects was very similar. Five subjects (classified as Group I in Table 1) showed expected postural, tapping, and biting records; however, during swallowing and sucking, these subjects showed greater contractions in the orbicularis oris muscles than in the mentalis muscles. Sixteen subjects (classified as Group II) showed dominance of the mentalis muscles during swallowing and sucking. These 16 also had action potentials manifested in the mentalis during tapping and biting, and 8 of them even had mentalis contractions in postural position (those who could not relax that muscle when their mandible was at rest). The other 3 subjects (Group III) showed a mixture of mentalis and orbicularis

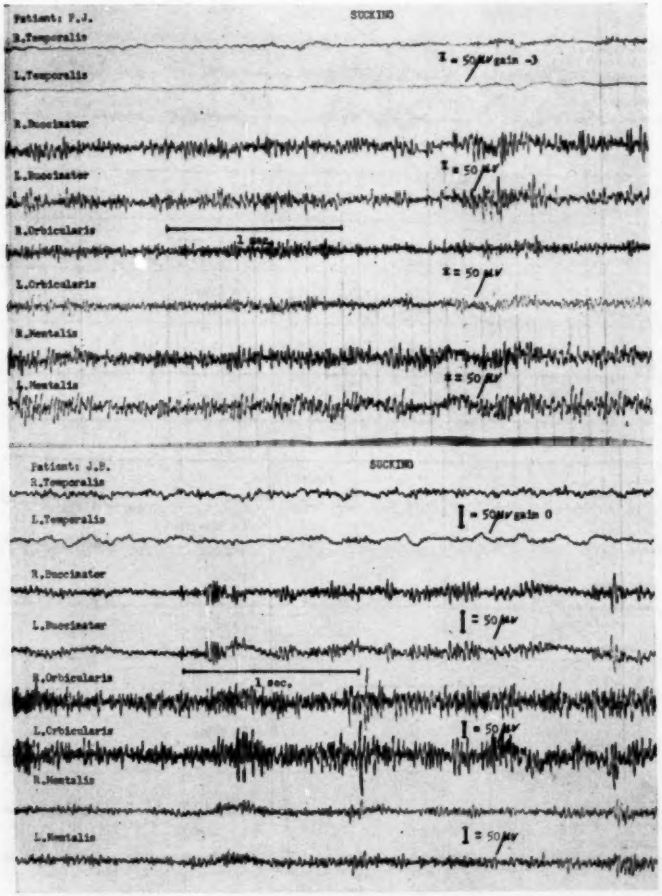

FIG. 9.—Electromyograms of two subjects during sucking. *A* shows a sucking pattern with preponderance of the mentalis muscles. *B* shows the dominance of the orbicularis oris muscles. Note also the minimal contraction of the temporalis muscles in both cases.

oris dominance. These were classified in a separate group so as not to confuse the analysis of the rest of the sample.

An attempt was made to correlate all the data collected, not only with the electromyographic findings, but also with each other. There was no correlation found between these findings and Cook's study.

The over-all amplitude of contraction, the presence of cross-bite, and the finger positioning also showed a complete lack of correlation with any data.

Table 1 gives the dental and skeletal values of the sample. In the fourth column, "B" pt. to "A" plane, one finds 7 Class II's according to the Jenkins' analysis; they

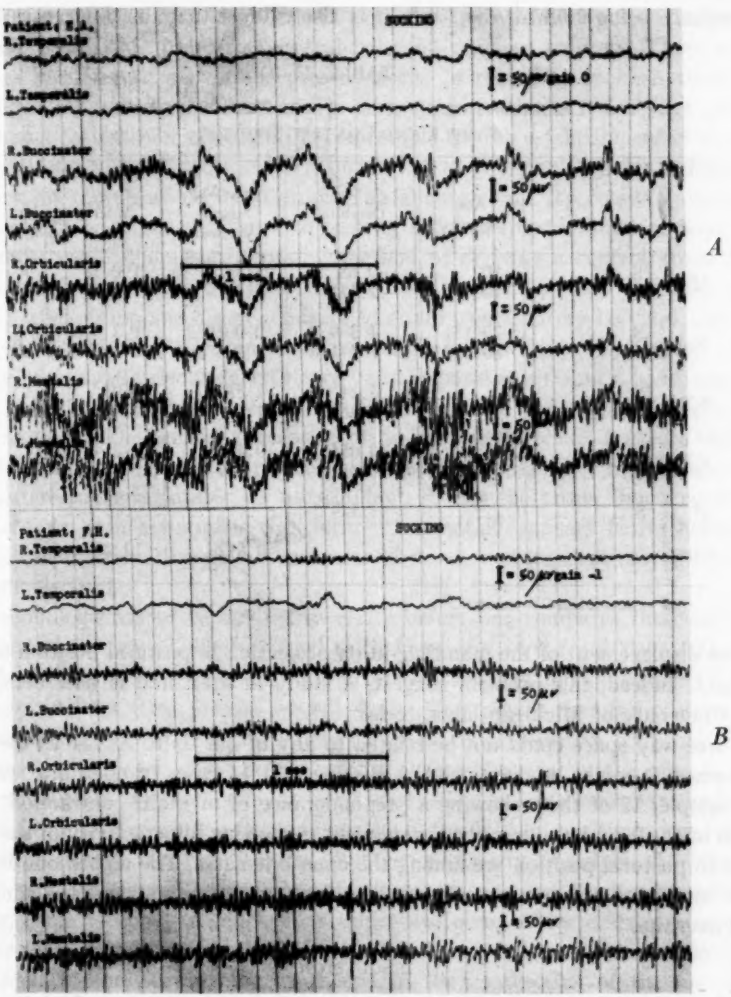

FIG. 10.—Electromyograms of two subjects during sucking. Both *A* and *B* show the mentalis muscles dominant. *A* has a certain rhythm, absent from the *B* records. Note again the minimal contraction of the temporalis muscles.

are indicated by an asterisk and were so classified when the given value was more than two standard deviations away from Jenkins' mean for "normal" occlusions. It is to be noted that 6 of these 7 patients are visceral swallowers. The remaining 4 visceral swallowers were skeletal Class I, three being dental Class II's and only one Class I.

A control group was studied in order to assess the validity of the numerical values collected on the sample. The control group was taken from the files of the Elementary School Growth Study, University of Michigan, and paired by age with the sample. It is shown in Table 2 that the overbite and vertical free-way space differences between the sample and the control group are not statistically significant. In fact, these values are, respectively, at the 20 and 50 per cent level of confidence. The 1 per cent level of confidence being 2.68 for the *t*-test, it is also evident that the differences in antero-

TABLE 2

CEPHALOMETRIC VALUES OF THE THUMB-SUCKING SAMPLE AND CONTROL GROUPS*

		Age (Months)	Overbite	Free-Way Space/ Vertical	Anteroposterior
Mean	S	102	−0.68	3.58	P 0.18
	C	100	−2.17	3.40	P 1.11
Range	S	70–131	−4.3–9.0	0.0–10.2	P 2.3 to A 2.4
	C	60–132	−7.4–1.6	1.0–8.4	P 3.9 to A 1.5
S.D.	S	.....	3.14	2.38	1.03
	C	.....	2.26	1.90	1.44
S.E.	S	.....	0.654	0.496	0.214
	C	.....	0.451	0.379	0.287
x^2	S	.....	2.529	.....	.....
t-test		.....	.....	0.612	12.23

* S = sample and C = control.

posterior displacement of the mandible in its projection to postural position are highly significant. Indeed, this problem suggests a study in itself and is now being studied in the University of Michigan laboratories.

The free-way space could not be related to any of our data. As far as the overbite is concerned, it might be mentioned in passing that 13 cases of open bite were found in the sample, 12 of them showing a preponderance of mentalis contraction.

Seven of the 9 subjects described previously as showing hyperactivity of the mentalis muscles in postural position are among the open-bite cases. The eighth one has a very shallow bite, with 0.5 mm. overlapping of the upper incisors, and the ninth has a normal overbite.

DISCUSSION

The significant observations in this study pertain to (1) thumb-sucking as it relates to malocclusion and (2) the neuromuscular complex of thumb-suckers as analyzed electromyographically.

In view of the several divergent interpretations of thumb-sucking, it is not surprising that, in a small sample like the present one, numerical values are not statistically significant. If one takes the overbite values, for example, the standard deviation (S.D.) and χ^2 tests deny any statistical significance (Table 2). If one looks at the range, however, —4.3 to 9.0 for the sample, and —7.4 to 1.6 for the control group, there is a tendency toward more positive values in the experimental sample. A range of 13 mm. for a sample of 24 subjects does not allow statistically significant conclusions. In order to validate the apparent trend observed, a larger sample would be required, and an electromyographic analysis of such a sample would necessitate a longer period of time than was available for this study.

No electromyographic study was done on a control group. Many actions other than sucking have been studied electromyographically by different workers. These studies will serve as partial controls for the present work. In regard to sucking, three non-sucking subjects have tried unsuccessfully to register a consistent sucking pattern. The repetitious nature of a habit itself is responsible for the establishment of a neuromuscular pattern; without habit, there is no *consistent* pattern. To ask a non-sucking child to suck his thumb would be to propose an abnormal act. To compare one of the numerous records he might produce with records obtained from a thumb-sucking child would be most misleading. An alternative method of deriving a control group might be to teach children how to suck their thumbs, but the comparison between a highly conditioned reflex group and a non-conditioned group would hardly be valid. One feels obliged to accept this limitation and recognize it when drawing conclusions.

Electrode placement, electrode efficiency, and apparatus efficiency have been discussed by many workers.[4, 12] One has to decide between the high selectivity of a needle electrode and the broader perception of the surface electrode. A needle electrode was used for the buccal wall, for it is felt that the great possibility of overlapping of fields in that region necessitated its use. Tulley[9, 15] uses the terms "buccinator" and "buccal wall" in both his studies. His term "circumoral" appears better because of the difficulty in fractionating the seventh cranial nerve muscles electromyographically with surface electrodes.

The methodology has to be studied carefully before one compares the conclusions of two different workers. Tulley[9, 15] used calibration of $\frac{1}{4}$ inch for 200 or 400 microvolts. Schlossberg[16] used $\frac{1}{4}$ inch for 100 microvolts. A deflection of $\frac{1}{4}$ inch (6 mm.) for 50 microvolts was used for the present study. This means that the present setup is eight times more sensitive than Tulley's and twice as sensitive as Schlossberg's. Perhaps this is the reason that certain activities were observed in the present study which did not appear to either Tulley or Schlossberg. The lack of findings in the buccal-wall region is more surprising when one considers the higher sensitivity used in this study. This is merely the repetition of divergence already found in the literature. Alderisio[17] and Winders,[18, 19] studying the intraoral pressure changes involved in swallowing, came to opposite conclusions. Alderisio found an increased pressure buccally to the molar region during swallowing, while Winders found the contrary. When one considers this pressure as the expression of the contraction of the buccal-wall muscles, the present work stands, on this point, with Winders as Tulley does with Alderisio.

It was also found that the orbicularis oris muscle can and does behave as two

separate muscles, right and left. A unilateral analysis can be accepted for a study such as this, but a precise analysis of the function of the orbicularis oris muscle would call for a bilateral analysis.

The frequent repetition of each exercise by the subjects was an assurance that a fair picture was being observed. Seven periods of 15 seconds each in the postural position, a minimum of 18 swallows, a minimum of 30 tappings and hard bites, and two periods of sucking with an average time of 66.7 seconds were each carried out for each child.

No two subjects ever gave identical records. Twenty-one subjects were remarkably consistent, and their classification is the fair image of their actual behaviors. Sometimes variation in amplitude was observed without altering the basic pattern of the contraction. Since this variation was seen both before and after sucking, it was not felt to be significant.

It was found that there is little change in the orofacial neuromuscular complex in the brief period following sucking; rather, the abnormal patterns seem so firmly established as to persist in the sucking subject. This phenomenon was found in every subject and seems to be of considerable clinical importance. The implication is that it may not be sufficient to correct the thumb-sucking habit alone if the abnormal orofacial muscular habit persists. One should then correct, or at least attempt to correct, the abnormal habitual muscular behavior in the circumoral region, as it will not disappear simultaneously with the cessation of the thumb habit. An increased contraction of the mentalis muscles is, in itself, sufficient to interfere with orthodontic treatment. Patients presenting an increased overjet with an increased mentalis contraction have a tendency for the lower lip to be pushed behind the upper incisors, preventing the reduction of the overjet or interfering in the retention.

An interesting side light to this study is the minimal contraction seen in the temporalis muscles during sucking. According to Carlsoö,[20] Jarabak,[21] Moyers,[5] Perry,[22] and others, the temporalis muscles usually show action potentials during the elevation of the mandible. As the temporalis muscles were the only fifth cranial nerve muscles studied, they were the ones used as the criteria to decide whether the subject was biting or not during sucking. It appears that no subject was biting on his thumb during sucking, as only minimal contractions were seen in the temporalis muscles. One question might be raised, though: Would the temporalis muscles show any contractions if the thickness of the thumb or the finger sucked was not forcing the mandible beyond the postural position? The hypothesis is offered that the free-way space is not responsible for the observed minimal contractions of the temporalis muscles, since it was highly variable among the subjects. Some subjects had negligible free-way space, and their thumbs were certainly forcing the mandible beyond its postural position. Even these subjects did not show other than minimal contractions of the temporalis muscles during sucking. It is questionable, therefore, whether the elevation of the mandible is responsible for the callus often seen on the sucked digit. Finally, in order to test this presumption further, three non-sucking subjects have sucked their thumbs, applying various biting pressures; action potentials in the temporalis muscles were found in every case.

It was thought that the findings of the English group on the swallowing behavior would have greater application in the present study. Rix[10] says that "poor oral habits

behaviour" (swallowing) often lead to a sucking habit. It is impossible to draw such a conclusion from the data derived in the present study, as the sample is divided almost equally into three groups. It is impossible to designate the swallowing habits as a cause of sucking habits.

Rix,[10] and Gwynne-Evans[23, 24] also associate visceral swallowing patterns to overjet, cross-bite, etc. Table 3 indicates why this study does not hold the same conclusions.

Too many malocclusions in this sample were not associated with visceral swallows. In fact, the mean overjet of the visceral swallowers is less than the mean of the whole sample. Four out of 8 cross-bites were visceral swallowers, and 5 out of 12 open bites were also visceral swallowers. Furthermore, similar general orofacial muscular behavior was seen in somatic swallowers as in the visceral swallowers when not swallowing. It is therefore impossible to establish a cause-to-effect relationship when 21 subjects have similar general facial muscle behavior except in the act of swallowing. The clinical implications are, however, the same; regardless of cause or effect, a muscular rehabilitation is necessary.

TABLE 3

DENTAL FINDINGS FOR VISCERAL SWALLOWERS

Visceral Swallowers	Overjet	Cross-Bite	Overbite
J.V.	11.0	.....	−1.0
L.B.	5.9	X	0.0
G.F.	8.3	X	0.0
N.H.	6.2	.....	−1.0
F.H.	5.6	.....	−3.0
P.J.	3.6	X	0.0
D.L.	7.5	.....	0.0
C.P.	5.9	.....	−4.1
E.T.	7.2	.....	−2.9
E.B.	2.3	X	+1.3
Mean...	5.35	.....	.....

Moyers[2] and Schlossberg[16] have found that in Class II subjects the mentalis muscles were more active than in Class I subjects. Both experienced difficulties in inducing relaxation of this muscle in the postural position. Their findings were probably less dramatic than the present one because they were both working with a sensitivity of 100 microvolts for $\frac{1}{4}$ inch. Of interest in the present study is the fact that we had as many Class I's as Class II's showing this abnormal mentalis pattern. It means, first, that the neuromuscular complex is not exclusively influenced by the genetic and skeletal development. Thus a child with an angle Class I malocclusion and a thumb-sucking habit might precipitate Class II symptoms if this abnormal circumoral behavior appears. It would also seem that, in treatment of a Class I sucking subject, one must consider the same possible interference from the mentalis muscles as he does with Class II subjects. Finally, in considering the older subjects (T.E., R.W., G.A.O.) who remained in a Class I molar relationship in spite of a Class II's neuromuscular pattern, one must admit that the abnormal neuromuscular behavior, as well as the sucking habit, did not cause a definite malocclusion.

In concluding the discussion, the absence of a statistically significant relationship

between open bite and the rest of the data does not alter the fact that 11 out of 12 of the open-bite cases were found on subjects showing abnormal contractions of the mentalis. An explanation is that the open bite leaves a greater space to be sealed in the acts of swallowing, whistling, etc. The mentalis muscle would help to seal the lower lip against the upper. The relative importance of lower lip and mentalis muscle is difficult to ascertain, considering the actual stage of refinement of electromyography and the fact that the lip and mentalis muscles function together so synergistically. As a result of this research, it is felt that the seventh cranial nerve muscles form a physiological functional unit that is arbitrarily divided for clinical use. Perhaps treatment directed at the mentalis muscles might effect, secondarily or even primarily, the orbicularis oris muscles. Consideration of a single circumoral muscle is almost impossible.

SUMMARY

An electromyographic study of the temporalis muscles and some of the facial muscles was performed on 24 thumb- or finger-sucking children. A radiographic and cast analysis was included. Records of intraoral pressures developed by the subjects during sucking were also available. Twenty-one subjects showed remarkable consistency in their muscular behavior. The dominant muscle was either the orbicularis oris or the mentalis. The present findings in regard to swallowing were identical with Tulley's. There is some disagreement, however, with the observations of Rix and Gwynne-Evans. The muscular behavior was found not to be altered in the short period following sucking, but rather the abnormal patterns appeared to be firmly established and to persist.

The overbite values were the numerical values showing the greatest interest, without, however, being statistically significant. Overbite seems to be related to hyperactivity of the circumoral musculature. Abnormal behavior of the mentalis previously associated with Class II division I malocclusions was, in the present study, found on any skeletal or dental malocclusion.

This study has emphasized the thought that the seventh cranial nerve muscles form a physiological functional unit and cannot easily be isolated electromyographically. It finally reflects the theory that malocclusion caused by thumb-sucking is more a matter of individual response to stimuli than a highly classified cause-and-effect syndrome.

CONCLUSIONS

1. Orofacial neuromuscular behavior is highly individualistic. One cannot assign a cause-effect relationship between the thumb habit and muscle pattern.

2. The neuromuscular behavior could not be related to the skeletal or dental aspect of the malocclusions.

3. The callus often seen on fingers of sucking subjects does not seem to be caused by mandibular elevation but by the pressure of the digit against the teeth.

4. There was no observable relationship between the retained visceral swallow and thumb-sucking.

5. There seemed to be no relationship between the pressure applied by the thumb and the neuromuscular behavior.

6. The abnormal mentalis contraction found in 19 subjects suggests that special

early attention should be given to children displaying such contraction in conjunction with a sucking habit. The control of this muscle might be considered as preventive orthodontic therapy, and correction of the abnormal contractions persisting after orthodontic therapy would seem indicated.

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Effect of Salivariadenectomy and Administration of Salivary Gland Homogenates upon the Reproductive Organs of the Female Rat

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In addition to the well-known functions of the salivary glands—that is, secretion of the saliva that aids in the mastication and swallowing of food, digestion of starches, protective functions, etc.—some early evidence (1940) from the work of Lacassagne and Chamorro[1, 2] indicated that these glands may be associated with the endocrine system. These investigators found that castration or hypophysectomy in the male mouse produced pronounced atrophy of the serous tubules of the submaxillary gland. These findings have since been supplemented by other investigators[3-6] reporting similar and related observations in the rat. Also in 1940, Higashijo[7] claimed that distinct histologic changes appeared in the hypophysis, parathyroids, thyroids, and bones of salivariadenecomized rats (parotid glands only ligated). He also claimed that the testes, thymus, and spleen were atrophic, while the suprarenal cortex and uterus were hypertrophic. These findings would seem to suggest a possible endocrine function of the salivary glands themselves. It should be pointed out, however, that, in this experiment, control and operated animals were receiving 10 mouse units of estrone per gram of body weight twice a week.

In 1942, Ginn and Volker[8] published an interesting paper in which they described a rusting condition of the fur and a significant reduction in the rate of growth in female desalivated rats. They suggested a possible disturbance in the metabolism of the sex hormones, since rusting of the fur had been shown to be related to such a disturbance.[9]

More recent evidence has lent considerable credence to the idea that the salivary glands possess an endocrine function. Generally, this later experimentation has developed along two separate lines: the attempt to relate salivary gland to thyroid gland function,[10-18] and the attempt to relate salivary gland function with reproductive organ or adrenal function.[7, 19-22] In view of some contradictory evidence and diversified conclusions concerning possible salivary gland–thyroid gland interfunctions[11, 12, 14, 16] and what seemed like the more solid experimental evidence supporting an endocrine action of the salivary glands upon the female reproductive organs as the target organs, it was decided to attempt to resolve this problem by pursuing this latter course.

Taking this experimental line, the investigations of Bixler, Webster, and Muhler[20, 21] seem of special significance. They studied the effects of salivariadenectomy on the reproductive organs of the female rat. Performing operations on weanling rats produced re-

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tarded development of the reproductive organs. They concluded that this effect was not entirely nutritional, since animals which were pair-fed with the operated animals showed no ovarian or uterine changes. A bioassay of the gonadotropin content of the pituitaries of salivariadenectomized rats indicated that the operation resulted in an increased amount of both FSH and LH in the pituitary glands. From these results they suggested that removal of the salivary glands caused inhibition of the release of gonadotropic hormones from the adenohypophysis, which would account for the retarded development of the reproductive organs.

As will be noticed from the evidence presented in the early publications making the salivary glands suspect of endocrine function, little has appeared in the literature pursuing resolution of this matter, except for the work of some Japanese investigators,[23-32] whose works, however, seem to lack confirmation by other investigators. Still, as far as is known, the Japanese investigators have not attempted to correlate reproductive organ activity with the salivary glands. Inasmuch as it is this aspect which became the primary interest of this investigator, their work is not discussed here but is summarized later in this paper.

From available literature on this subject, it is clear that the various authors have suggested endocrine functions for these glands after noting what happened to the animal and to various target organs only after salivary glandular extirpation. Inasmuch as these glands have not only an exocrine function that aids the animal in the actual eating and swallowing of food but an exocrine secretion that also contains digestive enzymes, some of these experiments may have been open to criticism on the grounds that removal of the salivary glands actually interfered with the nutrition of the animal. Also, if the salivary glands do possess an endocrine function, then it must be postulated that the glands themselves are the sites of a production, modification, or storage of some endocrine substance.

With these thoughts in mind, it was decided to study the effects of salivariadenectomy on the reproductive organs of the female rat compared with the effects in salivariadenectomized animals injected with homogenates of salivary glands.

EXPERIMENTAL METHODS

Forty-two homogeneous female rats of the Holtzman strain were used. At the beginning of the experiment, these animals were approximately 75 days of age with a mean weight of 160 gm. Throughout the experimental period they were maintained on the standard Rockland diet and water ad libitum.

For purposes of the experiment, they were divided into three groups of fourteen animals. These were designated by Group C (for control), Group O (for operated), and Group OH (for operated, homogenate-treated).

Animals of Group O were salivariadenectomized and thereafter remained untreated until sacrifice. Animals of Group OH were also salivariadenectomized, but began receiving homogenates of salivary glands after the surgery and continued under this treatment until sacrifice. Animals of Group C remained unoperated and untreated and served as controls.

For purposes of giving the homogenate injections to Group OH, the animals were divided into four subgroups. These subgroups were designated 1, 2, 3, and 4, consisting of three, four, four, and three animals, respectively. Later, the other two groups were

also divided into corresponding subgroups, which correlated time of sacrifice with animals of Group OH. As will be described, this division into subgroups facilitated the preparation and injections of standardized dosages of the homogenates.

The method of preparing and injecting the homogenate was carried out in the following manner. If, for instance, the four animals of subgroup 2 were to be injected, four mature female rats were sacrificed, and their salivary glands were removed. The glands were immediately cut up into small pieces and placed in a mortar with a small amount of sand and 0.9 per cent sodium chloride. The mortar and pestle, sand, and saline solution had been kept in a refrigerator up to this point. The tissues were then thoroughly ground for 5–10 minutes. After grinding, enough 0.9 per cent sodium chloride was added to bring the mixture to the desired concentration. The mixture was then decanted off the sand layer, divided equally into four syringes, and injected intraperitoneally into the four rats in subgroup 2. Homogenates were prepared and injected in an identical manner for the three other subgroups, with the exception that the salivary glands of three animals were used for the subgroups of three animals. All animals were injected every fifth day. Therefore, each rat in subgroup 2 received an estimated dosage of homogenate reducible from the complete set of salivary glands from one rat every 5 days.

The subgroups were sacrificed in the order 1, 2, 3, and 4 after receiving seven, eight, nine, and ten injections, respectively. Therefore, a time interval of 15 days existed between the sacrifice of subgroup 1 and subgroup 4.

The same number of animals was taken from each of Group C and Group O, designated by the same subgroup number (1, 2, etc.), with the corresponding subgroup being sacrificed with those of Group OH.

Since each animal had received its first injection 2 days after surgery, the time of sacrifice for each subgroup recorded in days after surgery would be 32, 37, 42, and 47 days, respectively.

At sacrifice, all gross animal weights were recorded, and the reproductive organs were removed and fixed in formalin. Gross autopsy was performed, and the ventral-median neck area of the surgically treated animals was examined to determine completeness of removal of salivary gland tissue. In almost half the cases, small remnants of parotid gland tissue were grossly visible. Owing to the diffuse nature of this gland, complete surgical removal would have been possible only by excessively traumatizing the animal. Also, it should be pointed out that the rat has two pairs of sublingual glands, those that lie in close proximity to the submaxillary gland and which were removed and another pair situated under the mucosa of the floor of the mouth.[33] These are very tiny, compared with the rat's other salivary glands, and again it was felt that removing these would only accomplish excessive traumatization, especially since significant results of the type that this investigator had hoped to substantiate further had already been accomplished without their removal.[20, 21]

RESULTS

Several methods were used to determine whether there was a response in the reproductive organs of these animals that could be attributed to salivariadenectomy and the injections of homogenates.

The ovaries were weighed by a method described and recommended by the British *Pharmacopoeia* (1948) for bioassays of gonadotropins.[34] In this method, the ovaries

are fixed in a solution of formaldehyde, transferred to 70 per cent alcohol, and weighed after a constant short period of drying on filter paper. The advantage of this method is that an accurate and careful removal of fat and hilum from the ovaries is possible without the concomitant fear of the drying, crushing, or tearing which may occur with fresh tissue—tissue from which slides are to be made. The ovaries were also examined under a dissection microscope. Mature follicles and corpora lutea were counted. The ovaries and uteri were then sectioned and examined microscopically to determine activity and size as measured by an ocular micrometer.

The results of these studies shown on the following pages in photomicrographs and tables not only verify findings of other authors that salivariadenectomy in the female rat is followed by atrophic and degenerative conditions in the reproductive organs[20, 21] but also add considerable support to contentions that the salivary glands of the rat are involved in an endocrine activity, by the finding that intraperitoneal injections of salivary gland homogenates not only completely reverse the atrophic conditions in the reproductive organs but actually increase the activities of these organs above the control level.

TABLE 1

OVARIAN WEIGHT

	Rats	Ovarian Wt. (Mg.)	*t*-Value
Control (C)	14	38.3±7.2*	2.89(O)
Operated (O)	14	29.7±7.8	4.79(OH)
Operated (OH), hom.-treated	14	45.8±9.2	2.30(C)

* This is the standard deviation.

Since this was to be the first known attempt to determine an animal's response to injections of salivary glandular homogenates, it was decided to accept the 0.10 level of significance as indicative of true differences in the given parameters. However, as it turned out, the most important parameters were significant well beyond the 0.05 level.

The mean ovarian weight of the control animals was 38.4 mg. The ovaries of the desalivated animals were represented by a mean of 29.7 mg., while the ovaries of the desalivated animals that received homogenates averaged 45.8 mg. Therefore, of the twenty-eight salivariadenectomized rats, the mean ovarian weight of the fourteen animals that received salivary gland homogenates represented a 54 per cent increase in mean ovarian weight over the fourteen animals that did not receive the homogenates. This difference was significant beyond the 0.01 level. The difference between the means of the control and operated groups representd a 23 per cent decrease in ovarian weight of the operated animals, which was significant beyond the 0.02 level. The mean weight of the ovaries of the animals in the operated homogenate group represented a 19 per cent increase in weight over the mean ovarian weight in the control group and was significant beyond the 0.05 level.

From these figures, corresponding levels of activity in the uteri of these animals would be expected, which was the case. These were not weighed, but response was quantitatively determined by examination of sections microscopically by means of an ocular

micrometer. All parts of these organs responded greatly to the ovarian hormones. However, the endometrium and the size of the lumen vary drastically during the 5-day estrous cycle, while the myometrium does not show a great variation during cycling but will show the effects of variations in hormonal activity over a longer period of time (e.g., castration, pregnancy). Taking this point into consideration, it was decided to measure the thickness of the myometrium of all animals. In the controls the thickness of the myometrium was represented by a mean of 23.2 units. This parameter in the operated-homogenate group was 23.5 units. In the untreated desalivated animals, the mean uterine muscle width was 18.7 units. The difference between the means of Group OH and Group O was significant beyond the 0.05 level.

TABLE 2

THICKNESS OF MYOMETRIUM

	Rats	Thickness of Myometrium*	*t*-Value
Control (C)	14	23.2±5.1	2.12(O)
Operated (O)	14	18.7±5.4	2.47(OH)
Operated (OH), hom.-treated	14	23.5±4.4	0.24(C)

* 70.0 units = 1 mm.

TABLE 3

DIAMETER OF UTERUS

	Rats	Diameter of Uterus*	*t*-Value
Control (C)	14	214±76	2.13(O)
Operated (O)	14	160±51	2.09(OH)
Operated (OH), hom.-treated	14	206±61	0.29(C)

* 70.0 units = 1 mm.

In attempting to quantitate total uterine response, cross-sections were made of all uteri, and the total diameter of each uterus (one horn) was measured. This was done because each component of this organ (myometrium, lumen, etc.) will increase or decrease in size in direction proportion to any other component in response to total ovarian hormonal activity.[35, 36] These measurements showed a mean of 214 units for the control animals, 206 units for the operated homogenate group, and 160 units for the untreated desalivated animals. Since the diameter of this organ varies tremendously (more than doubling during proestrus and early estrus due to distention of the lumen with secretions of the glands) during the normal estrous cycle, the standard deviations of the diameter means are quite high (control, 77; operated, 51; operated-homogenate, 61). In fact, if very large groups of animals are used, the standard deviations of uterine diameters should be a reliable indication of whether normal estrous cycling is occurring. However, these high standard deviations prevented large differences between the mean diameters from being highly significant. The differences between the control and un-

treated desalivated animals and between the treated desalivated animals and untreated desalivated animals were significant beyond the 0.10 level.

A qualitative evaluation of these changes was made by histologic examination and examination under a dissection microscope. In the case of the ovaries, this revealed changes in folliculogenesis of the three groups that correlated well with the organ-weight changes. In both the control and the homogenate-treated salivariadenectomized groups, numerous follicles were observed in all stages of development. Total numbers of follicles were greater in the OH group; however, there did not appear to be so many mature follicles with the characteristic preovulatory papillae in this group as in the controls. Another observation that should be mentioned along with this was that the OH group produced three animals with ovaries that contained follicular cysts.

The ovaries of the untreated salivariadenectomized animals contained approximately 50 per cent less total follicles than did either Group C or Group OH. Mature follicles were observed, but most of these appeared to be undergoing atresia. Greater numbers of non-functional corpora lutea were found in this group. On the whole, the ovaries of this group were not so well vascularized, and the interstitial tissue was underdeveloped.

The qualitative histo-appearance of the uteri of the control and homogenate-treated, operated animals did not differ significantly in any respect. Both groups revealed uteri in all stages of the estrous cycle. The extent to which the uteri of both these groups differed from the untreated salivariadenectomized animals was evident from the time of sacrifice, when it was noted that only one uterus from the untreated-operated group was distended with secretions, while seven uteri showed this in the treated desalivated animals and six in the controls. This distention is characteristic of the proestrous and estrous phases. This was verified microscopically, where it was quite evident that the uteri of the untreated operated animals were atrophic. A few had atrophied so markedly that they appeared similar to the secondary sex organs of a castrate (Fig. 4).

Mention is made here of the gross weight changes observed in these animals, although these changes were not statistically significant, chiefly because of weight gain observed in the homogenate-treated desalivated animals—a gain that even carried over the control animal weights. This is worth reporting, since all experimental works found in the literature in which the salivary glands have been removed have always reported a weight loss in the operated animals.[8, 13, 20, 21] This has occurred even under pair-feeding conditions. However, the animals in this experiment were fed ad libitum, and a slight weight gain was still recorded. The mean body weights were as follows: control, 218.2 gm.; operated, 215.9; operated-homogenate, 226.1 gm.

No significant differences were observed corresponding to differences in the number of injections or in the times of sacrifice in Group OH.

DISCUSSION

The results presented here (along with findings of other investigators[20, 21]) seem to establish that there is a substance normally present in the salivary glands of the female rat which, upon removal by salivariadenectomy, causes retarded development and atrophy of the reproductive organs. Furthermore, this investigator has found that these changes can be completely reversed by injections of homogenates of the salivary glands.

From these findings, it could have been hypothesized that the actual reproductive

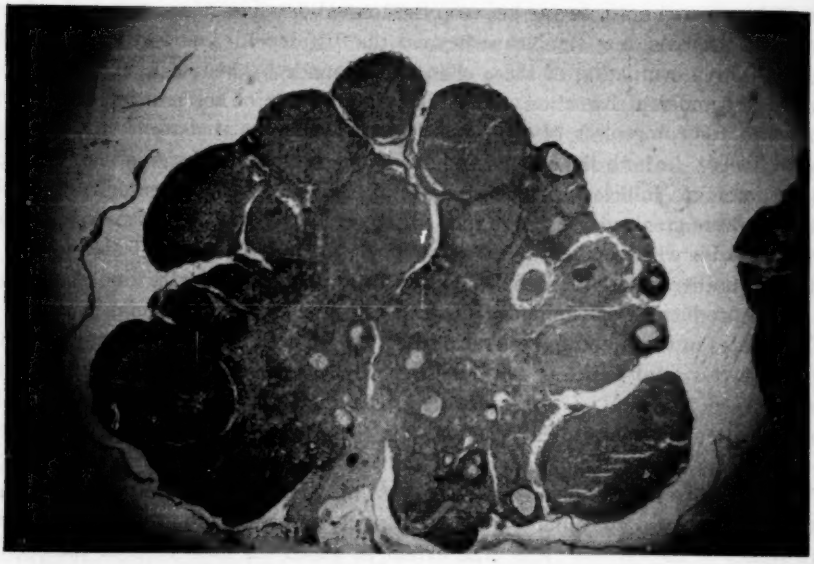

FIG. 1.—Section through ovary of salivariadenectomized animal which received homogenates of salivary glands. (Orig. mag. ×15.)

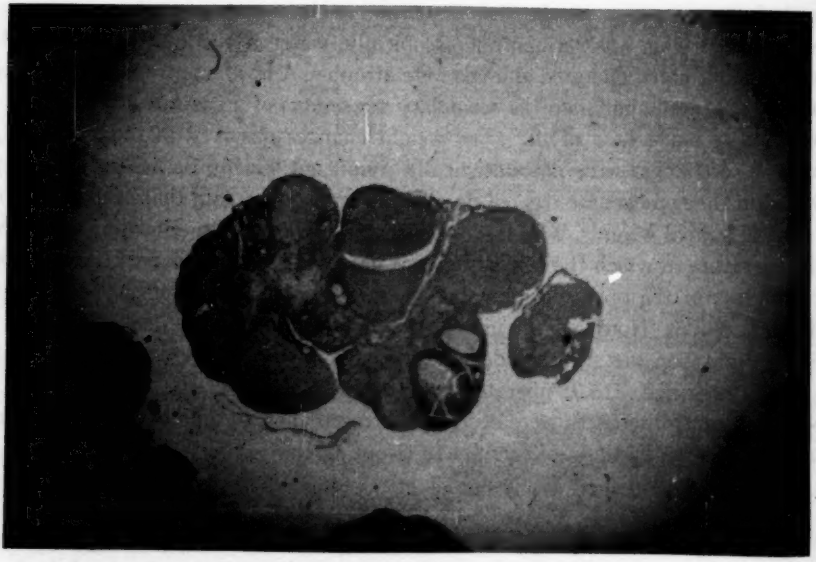

FIG. 2.—Similar section of operated animal which did not receive homogenates. (Orig. mag. ×15.)

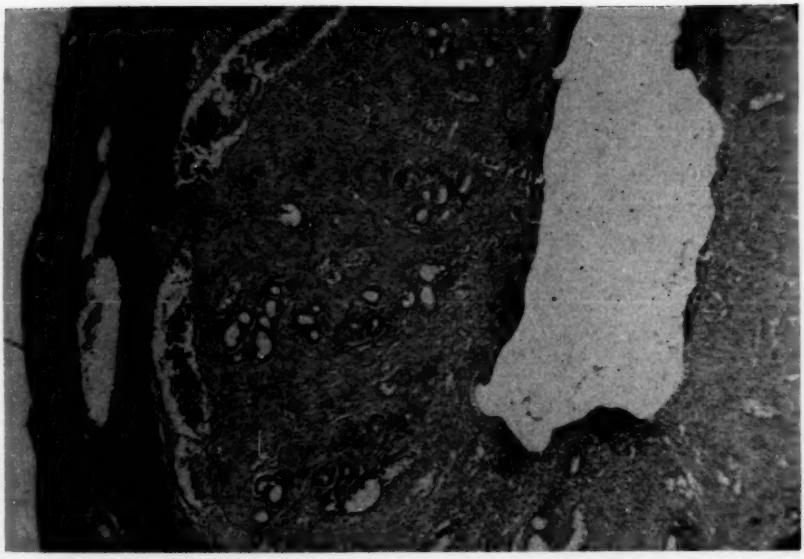

FIG. 3.—Cross-section through one horn of uterus of salivariadenectomized animal which received homogenates of salivary glands. Compare vascularity, thickness of myometrium, and size with Fig. 4. (Orig. mag. ×35.)

FIG. 4.—Similar section through the uterus of untreated, operated animal. This uterus closely resembled that of a castrate. (Orig. mag. ×35.)

FIG. 5.—Cross-section through one horn of uterus of control animal. Uterus is in diestrus. (Orig. mag. ×35.)

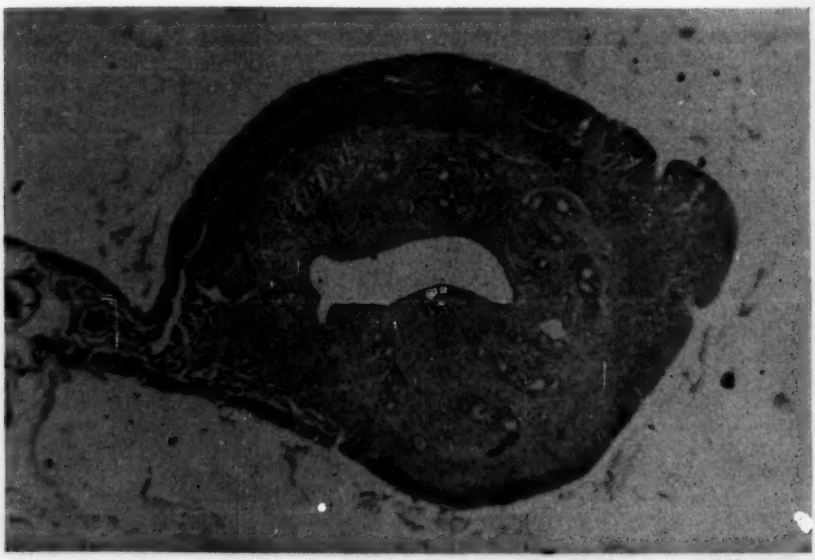

FIG. 6.—Similar section of untreated, operated animal in the diestrus state. This uterus, too, resembled that of a castrate. (Orig. mag. ×35.)

FIG. 7.—Cross-section through one horn of uterus of desalivate homogenate-treated rat that is obviously in the estrogenic phase. (Orig. mag. ×15.)

FIG. 8.—Similar section of uterus of untreated, operated animal. This section is identical with Fig. 6, except that Fig. 6 was taken at a higher magnification.

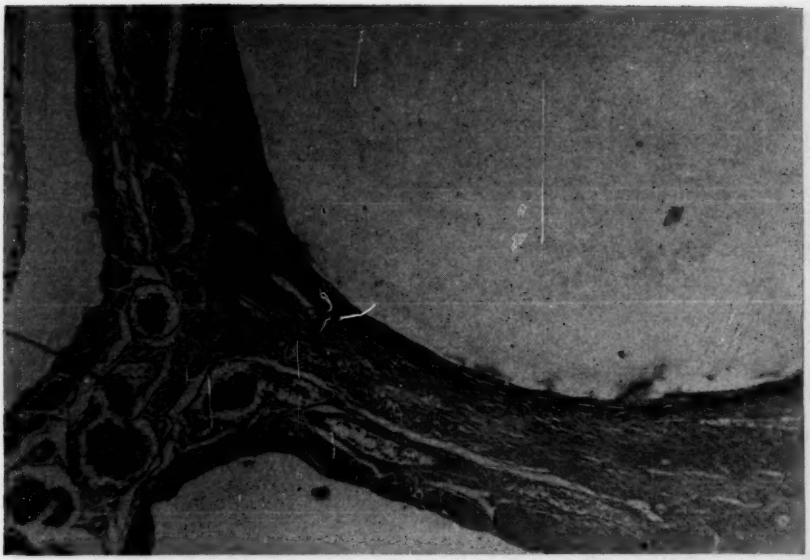

FIG. 9.—Cross-section of one horn of uterus of operated, homogenate-treated rat. This uterus is in the estrogenic phase. Note great distention and vascularity. (Orig. mag. ×35.)

FIG. 10.—Higher magnification of uterus shown in Fig. 9. (Orig. mag. ×15.)

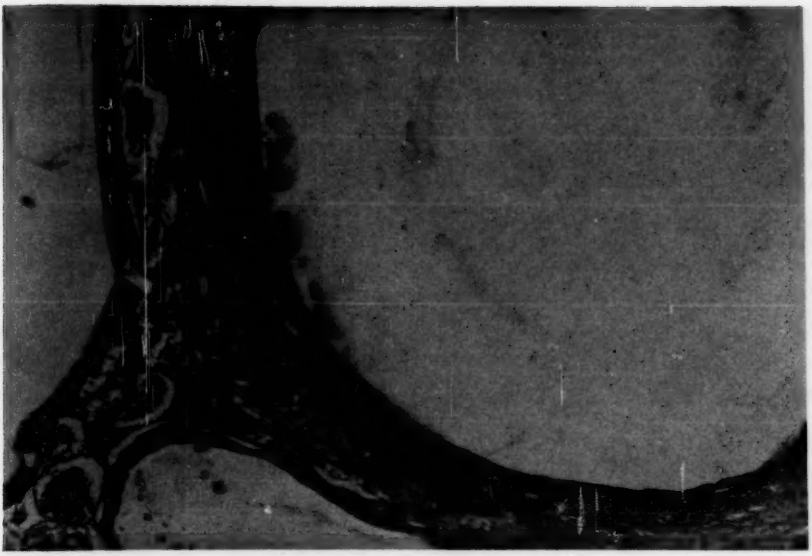

FIG. 11.—Part of wall of uterus of control animal in the estrogenic phase. Note similarity to uterus of operated, homogenate-treated animal in Fig. 9. (Orig. mag. ×35.)

FIG. 12.—Cross-section of one horn of uterus of untreated, operated animal. (Orig. mag. ×35.)

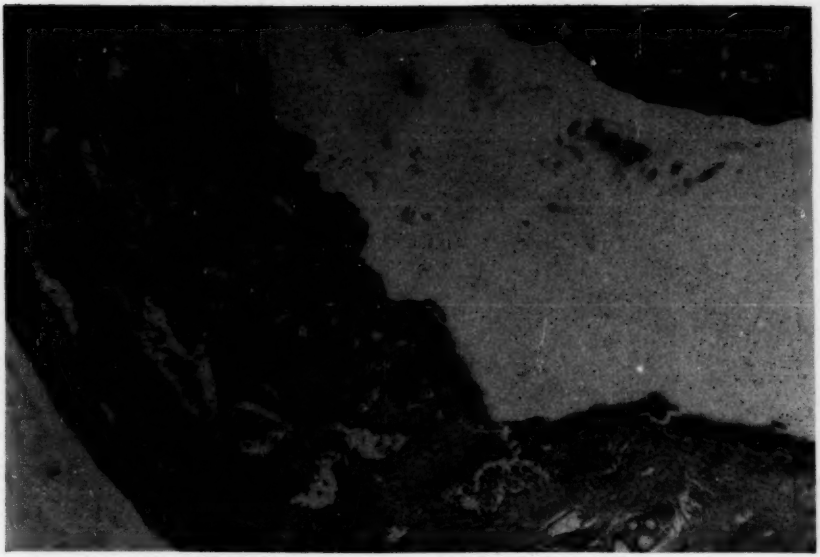

FIG. 13.—Uterus of operated, homogenate-treated animal in the metestrus stage. Note vascularity. (Orig. mag. ×35.)

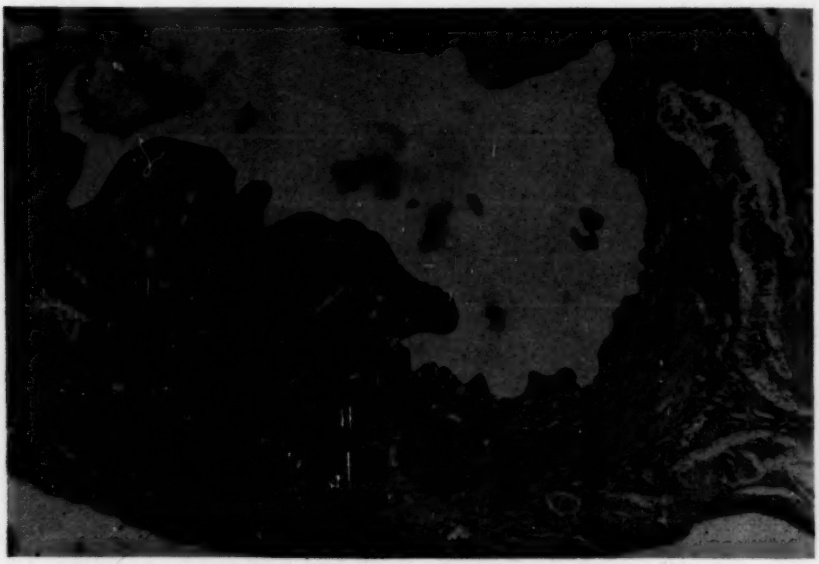

FIG. 14.—Similar section through uterus of control animal in the metestrus stage. (Orig. mag. ×35.)

ability of desalivated rats would be subnormal and, conversely, that reproductive ability could be restored by injections of the salivary gland homogenates.

In a paper published after the investigations in this paper were under way, Dimitri Afonski[19] has found one of these hypotheses to be true. That is, he found a very significant reduction in the ability of desalivated female rats to become pregnant. He also found a significant reduction in the ability of desalivated male rats to impregnate normal females. Unfortunately, he did not attempt restoration of the reproductive ability of desalivated animals with salivary gland homogenates.

Another recent publication of interest, the results of which emphasize one of the results of this paper, is that of Shaw and Wollman.[37] These investigators claim that desalivated rats actually eat more food and drink more water than controls, even though their growth rate is significantly less. It seems that it had always been more or less universally assumed that desalivated rats eat less than controls. This emphasizes the result of this investigation, in which the homogenate-treated, desalivated rats gained more weight than the controls.

TABLE 4

FINAL BODY WEIGHT

	Rats	Final Body Wt. (Gm.)	*t*-Value
Control (C)	14	218.2± 9.0	0.32(O)
Operated (O)	14	215.9±18.3	1.52(OH)
Operated (OH), hom.-treated	14	226.1±11.7	1.93(C)

The next question is how the substance that was present in the salivary gland homogenate was mediated, that is, whether it acted directly on the reproductive organs or through the pituitary gland. Of course, resolution of this problem calls for an experiment similar to the one described here, involving the adenohypophysis as the target organ, either directly through histologic studies and bioassays or indirectly by using hypophysectomized rats.

Although discussion on this point is somewhat speculative, it is also important—important because such a discussion elaborates ramifications of this problem yet to be elucidates and questions still be be answered; it also brings out ways by which these may be done. With this rationalization, we can proceed.

There is some experimental evidence supporting the pituitary route, as was mentioned previously.[21] (The results of Bixler *et al.* indicate inhibition of the release of gonadotropins from the pituitary after the salivariadenectomy.) The results of another experiment should be mentioned which, when considered altogether with results of investigations that have attributed the effects on growth and the reproductive organs to the salivary glands, allows speculation to proceed even further. This is the experiment by Wase and Feng,* who found increased uptake and delayed turnover of iodine 131

* The Wase and Feng paper seems to be the only one in the literature that has attacked possible thyroid–salivary gland relationship from the point of view of pituitary gland mediation. However, a comparative abundance of papers has appeared attempting to establish, or disprove, a relationship between these glands. These papers have taken an entirely different experimental approach by claim-

by the thyroid in salivariadenectomized mice.[17] Administration of thyroid-stimulating hormone (IP 5 μg.) restored the activity of the thyroids. Their conclusions were that the salivary glands play an important role in the regulation of the activity of the thyroid gland, probably by controlling the production and/or utilization of the thyroid-stimulating hormone.

Now a general picture emerges. This is the possibility that salivary gland extirpation causes general inhibition of anterior pituitary function. Logically following this possibility would be the assumption that injections of salivary gland homogenates could stimulate pituitary gland function above normal physiologic levels. As mentioned before, this suggests experimentation along this line with the anterior pituitary as the target organ for histologic studies and bioassays in salivariadenectomized animals and animals receiving salivary gland homogenates.

Although this is a new and startling biological concept, it must be admitted that what experimental results are available, even though scant, tend to indicate this possibility.

Another approach which could account for the results of this paper and others that it supports is the possibility of direct hormonal action upon the female reproductive organs by the salivary gland hormone substance. This hormonal action could be synergistic with the gonadotropins, which would explain the reproductive organ atrophy that occurs when either the pituitary or the salivary glands are removed. This approach could also be justified by a different interpretation of the results of the oft-mentioned Bixler *et al.* paper,[21] in which increased amounts of FSH and LH were found in the pituitaries of desalivated rats. As will be remembered, the authors' interpretation was that salivariadenectomy caused an inhibition of the release of these substances. Since no justification for this interpretation was presented in the publication, perhaps the anterior pituitaries of these animals were actually producing and releasing more gonadotropins in trying to compensate for the removal of the source of the synergizing salivary gland hormone. This interpretation would be more in agreement with the fact that castration in the rat produces increased blood levels of FSH and LH and increased amounts of gonadotropins in the hypophysis of these animals.[36, 38]

Other strong evidence supporting a direct action of the salivary gland hormone, although not upon the reproductive system, is the work of Yosijo Ito and his associates at the University of Tokyo, who, in attempting to prove the validity of the "salivary gland hormone theory," as proposed by Dr. T. Ogata,[30, 31] has isolated a crude protein from the bovine parotid glands which he has named "parotin."[23] The author finds that this substance enhances mineralization and calcification of bones and teeth.[23] The growth rate was increased in vivo, and the length of long bones was increased in tissue culture.[29] It was shown to reduce serum calcium levels in rabbits.[24] These investi-

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ing (and disclaiming) that the salivary glands function to control the thyroxine blood level by degrading or deiodinating thyroxine and thyroid analogues and recycling the iodide ion to the thyroid gland via the saliva and GI tract (Fawcett and Kirkwood, Ref. 11). The majority of experimental evidence, however, indicates that the salivary glands do not play an *essential* role in thyroxine metabolism by these means. This evidence is presented in a later paper by Fawcett and Kirkwood (Ref. 12) and in papers by R. W. Watts (Ref. 18), W. R. Ruegamer (Ref. 14), and W. Tong, G. D. Potter, and I. L. Chaikoff (Ref. 16). Mention of these facts was deemed appropriate here because this experimental approach will be one of the most frequently encountered in the literature in connection with a possible salivary gland hormone function.

gators also claim to have isolated a parotin-like substance in human urine[26] and a parotin-like substance in human saliva.[27]

Two investigators in the United States who have acknowledged their work have not been successful in attempts to confirm parts of it. (H. Fleming[39] reported that growth was not enhanced by administration of parotin; H. and M. Rosenberg[40] did not get a reduction in serum calcium level in rabbits upon administration of parotin.) This is not meant to suggest a negation of the Japanese authors' salivary gland hormone theory but to emphasize that two independent lines of research involving the same field have developed with few attempts at corroboration on either side. Hence, while recent investigators in the United States have only suggested possible endocrine functions of the salivary glands, Japanese workers were doing chemical studies of salivary hormone in 1944.[32] Therefore, although these workers have not reported any attempts to correlate reproductive organ activity or function with their salivary gland hormone, it must be admitted that a parotin-like substance could have been the active component or part of the active components of the salivary gland homogenates that produced the results reported in this investigation. This can easily be determined by conducting an experiment similar to the one presented here, but using both parotin and the complete salivary gland homogenate injections separately, and comparing response.

By now, it should be clear to the reader that the salivary glands must take part in important physiologic functions that are little known, not generally attributed to them, and seemingly unrelated to their functions that are well known—the exocrine. Moreover, the fertility of this field for investigation cannot be unnoticed.

SUMMARY

Salivariadenectomy in the female rat is followed by retarded development, decreased activity, and atrophy in the reproductive organs. These changes are characterized grossly by reduced organ weights and sizes; histologically, by the appearance of fewer mature follicles in the ovaries and lack of appearance of characteristic cyclic changes in the uteri. Vascularization of both the ovaries and the uteri of the desalivated animals was less than that of the controls. Injections of salivary glandular homogenates completely reversed these degenerative responses in the salivariadenectomized animals, increasing reproductive organ activity above the control level with the same criteria of response. Gross body weights of the homogenate-treated, desalivated animals also rose above that of the controls.

CONCLUSIONS

From these results, it must be concluded that the salivary glands of the female rat contain a substance that plays a role in the normal activity of the reproductive organs and, furthermore, can increase the activity of these organs above the normal. Moreover, this substance fits all requirements in the definition of a hormone.[36]

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A Continuous Culture (WiSh) Derived from a Human Gingival Carcinoma

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Various types of tissue cells in continuous culture have been found useful in virus and cancer research. Until now, three human oral cell lines have been propagated in serial tissue culture. These are as follows: KB strain from an epidermoid carcinoma of the floor of the mouth;[1] H. Ep. #3 strain from an epidermoid carcinoma of the buccal mucosa;[2] and a fibrocytic strain from a normal palatal mucosa.[3] This report concerns itself with a fourth oral cell culture (WiSh) isolated in January, 1958. It was derived from a human squamous-cell carcinoma of the maxillary gingiva.

CELL ORIGIN AND HOST HISTORY

W. S., a thirty-five-year-old white male, was first seen in July, 1957. Right maxillary swelling in the antral area and looseness of some molar and bicuspid teeth were the chief complaints. A biopsy specimen was diagnosed as anaplastic squamous-cell carcinoma. Surgical extirpation was attempted in December, 1957.

A fungating ulceration in the edentulous right central area and palpable cervical lymph nodes became evident soon afterward. On January 11, 1958, the gingival lesion was biopsied, and some teeth were extracted in preparation for radiation therapy. Half of the tissue was sectioned (Fig. 1), revealing an anaplastic squamous-cell carcinoma. The other half was immediately put into Hanks basic salt solution[4] for tissue culture.

Ionizing irradiation was delivered to the maxillary and cervical areas between January and March, 1958. At the end of treatment, the oral lesion had disappeared, and the cervical nodes were diminished in size. A chest film, however, revealed pulmonary nodules compatible with metastatic disease. The patient showed signs of intracranial metastasis in January, 1959, and expired one month later.

CULTURE PROCEDURE

The Hanks basic salt solution, into which the excised tissue was immediately placed, contained 100 units/cc penicillin and 100 μg/cc streptomycin to control bacterial contamination. The tissue was washed several times in this solution to remove excess red blood cells. Then it was transferred to a 60-mm. Petri dish for mincing with scissors to fragments of about 1.0 mm. The fragments were suspended in chick embryo extract and placed in Leighton tubes on cover glasses previously coated with chicken plasma. After clot formation, 1.5 cc. of medium, consisting of 20 per cent human serum in mixture 199 with $NaHCO_3$,[5] were added to each tube.

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OBSERVATIONS

Although the clots were almost completely liquefied, only a few cells were growing on the glass in 1 week. The medium was changed then, and subsequent changes were made weekly. The tubes with the best growth were maintained for 2 months. At the end of that time, one tube was trypsinized and subcultured to a 30-sq. mm. flask. The growth rate improved and has remained stable since. Mechanical detachment of the cells from the glass by scraping and washing has been preferred for subculturing because the cells are especially sensitive to trypsin. The WiSh cells have been serially

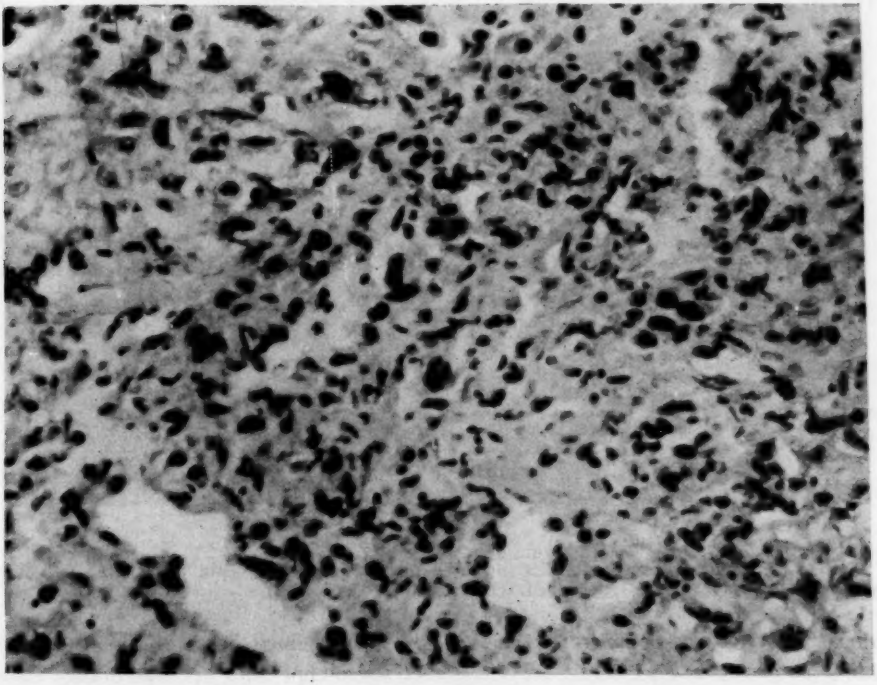


FIG. 1.—Biopsy section of tissue source for WiSh cell. Anaplastic squamous-cell carcinoma of maxillary gingiva. (Orig. mag. ×150.)

cultivated in twenty-one passages during 22 months and may be considered an established strain.

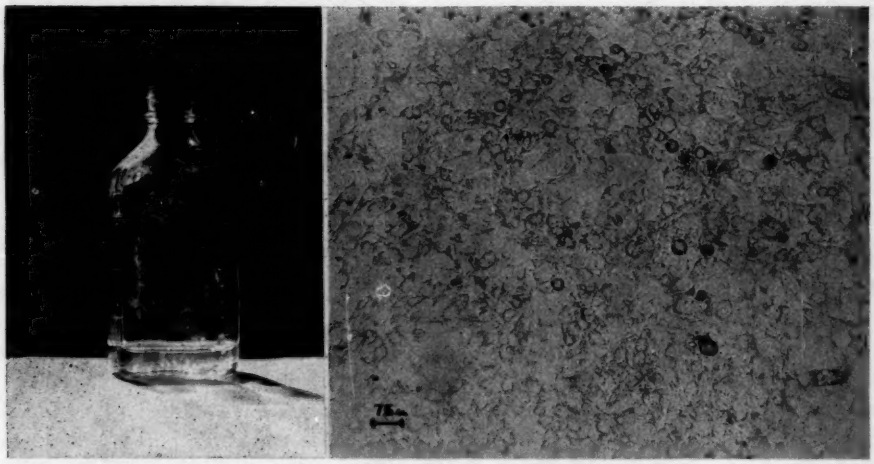
Comparison of serum volume effect on cell growth measured by nuclei counts indicates that 10 per cent serum retards growth and 40 per cent does not appreciably increase it. The type of media affects the morphologic appearance as well as the growth rate. The cells, like others,[6] are tolerant of acid condition but are affected adversely in an alkaline environment. The type and concentration of nutritional supplement to the media are also significant factors.[7]

MORPHOLOGIC APPEARANCE

In vivo (Fig. 2) the cells grow singly, attached to one another or in continuous monolayered sheets. If proliferation continues, the sheets become multilayered and

form colonies, which readily detach from the glass and initiate growth elsewhere upon settling. The cytoplasm, especially in old cultures, accumulates small granules that are refractile, usually densely packed, and not evident in stained preparations after ether-alcohol fixation. The granules have been identified as fat with Sudan IV stain following formalin fixation.

The morphologic similarity of the WiSh cell to HeLa[8] and other cell strains is evident in stained preparations (Fig. 3). The cytoplasm is characterized by stellate extensions, which, in some instances, resemble intra-epithelial bridges. Where growth is heavy, the cytoplasm is elongated and slender, with two or three tapering extensions. Polygonal morphology is apparent where cells are isolated. The cytoplasm is anom-



A *B*

FIG. 2.—*A*, WiSh cells growing on flat surface of prescription bottle. (Orig. mag. ×0.55.) *B*, Sheet of living cells growing on glass, unstained. (Orig. mag. ×120.)

alous, with a foamy appearance and often poorly defined borders. The cytoplasm emits a red fluorescence under the ultraviolet microscope with acridine orange stain. This evidence of RNA has been found characteristic of malignant cells when associated with morphologic atypia.[9] The ellipsoidal nuclei vary from 8 to 66 μ in diameter. Nuclear borders are irregular; moreover, they are in some cases extremely convoluted, as the nuclei appear to be undergoing an unusual form of budding. Chromatin clumping is rather prominent. The nucleoli are multiple and assume distorted shapes; some measure 10 μ in length. The nucleoli emit yellow-orange fluorescence under the ultraviolet microscope with acridine orange stain, which differentiates these RNA bodies from the DNA chromatin clumps. Few of the cells show actual mitotic formations.

During the cells' 1½ years of growth in continuous culture, changes in morphology appear to be a slight increase in nuclear size and a greater incidence of nuclei with the appearance of budding. The chromatin pattern seems to be more finely distributed, and the cytoplasm is more stellate.

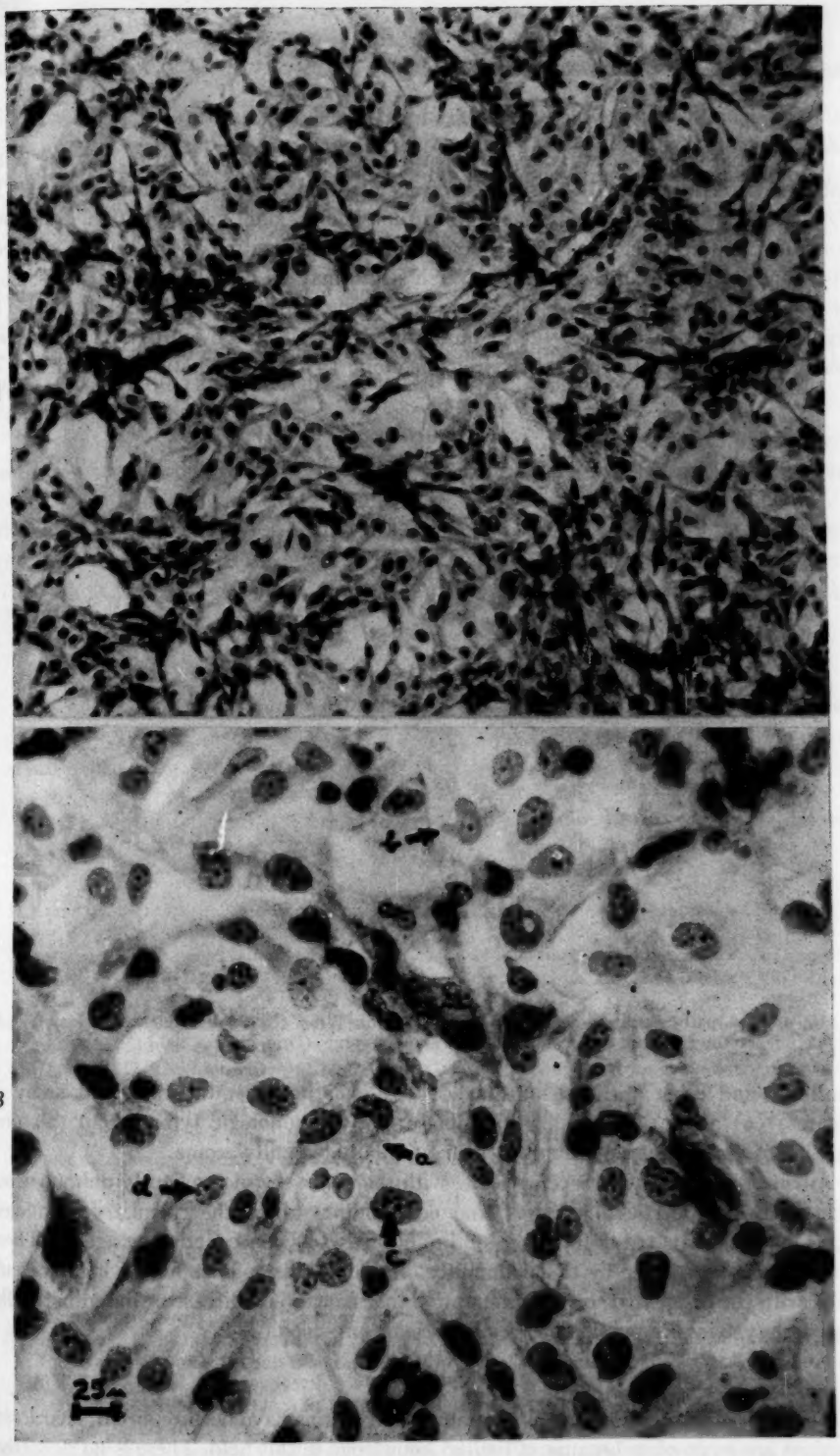


FIG. 3.—WiSh cell, May-Grumwald-Giesma stain. *A*, Note stellate appearance of cytoplasm. (Orig. mag. ×120.) *B*, Note foamy appearance and ill-defined borders of the cytoplasm (*a*); irregular nuclear borders which give the appearance of an unusual form of budding (*b*); multiple nucleoli (*c*); chromatin clumping (*d*). (Orig. mag. ×390).

VIRAL EFFECTS

No cytopathogenic effect is apparent with herpes or influenza viruses, but the WiSh cells are susceptible to APC, psittacosis, vaccinia, and Newcastle disease viruses. The cytopathologic effects (Fig. 4) include inclusion bodies, giant-cell formation, clumping, rounding, and destruction of cells. These results, demonstrating an interaction between virus and host cell, indicate that the WiSh strain may be useful in viral studies. Pure or standardized cell lines have already yielded basic information on the following: (1) tissue and cellular trophism; (2) metabolism of cells affected by viruses; (3) interference phenomenon with coexistent viral infection; (4) mode of action upon viruses of

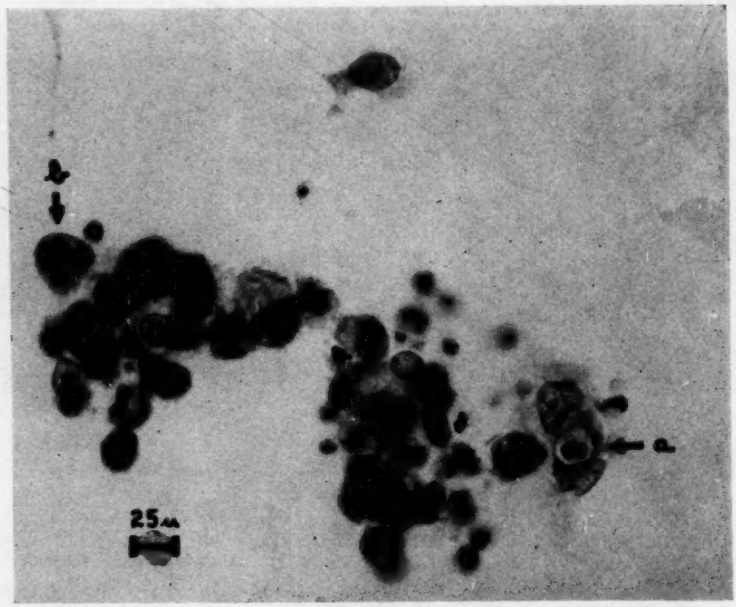


FIG. 4.—Cytopathogenic effect from vaccinia virus on WiSh cell. Notice nuclear inclusion body (*a*); tendency toward giant-cell formation (*b*); and clumping. (Orig. mag. ×390.)

antibody and other antiviral substances; (5) isolation, identification, and assay of viruses; (6) assay of neutralizing antibody; (7) immunologic typing; and (8) production of virus for use as complement-fixation antigen and vaccine.

Advances over the past several years, with the discovery of previously unknown human viruses, properties of viruses, and new findings about the chemistry of nucleic acids, have contributed to intensive studies of viruses as cancer-inducing agents. These studies have been aided by the availability of many kinds of human cells in tissue culture, with the result that, on the assumption that cancer is a disease of individual cells, new test systems for possible cancer viruses are provided.

DISCUSSION

Although many descriptions of continuous cell strains have been reported, characterizations are incomplete because of limited knowledge in this field. There is no definitive

differentiation between an epithelial cell and a fibrocyte. The conventional classifications used in histology do not apply to cultured cells. It is difficult, if not impossible, to differentiate between normal and malignant cells in culture.[10] This is due to the fact that malignant cells are generally recognized by morphologic criteria and cells in culture derived from normal tissue almost immediately assume and maintain malignant morphologic features. The lack of influencing homeostatic host factors may be the basis for this alteration. Observations after transplanting cultured cells to laboratory animals have allowed differentiation in some instances. Furthermore, there are changes with time in the cell characteristics of continuous culture strains.[11] Therefore, the key to the classification of cultured animal cells may be an integrative synthesis of biomorphology and biochemistry.

To solve this problem, data for the development of a systematic body of knowledge must be accumulated and analyzed. Advances in this field of scientific investigation,[12] which encompasses biophysical and biochemical cytology, have been slow because of the complexities and variations in cell systems and techniques. A variety of completely synthetic media must be devised and methods perfected to simplify tissue culture. Then results should be predictable and a classification of cultured animal cells effected. This is preliminary to the eventual practical applications of this phase of biology.

SUMMARY

The history of a continuous culture of a cell line (WiSh) derived from a human squamous-cell carcinoma of the maxillary gingiva has been reported. Preliminary observations on growth, morphology, and some virus susceptibilities have been described.

Appreciation is expressed to Miss Virginia Jentoft, whose guidance was indispensable in this work.

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An Explanation for the Hygroscopic Setting Expansion of Dental Gypsum Products

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Dental gypsum products when mixed with water and allowed to "set" in air exhibit an expansion of their peripheral boundaries that is known as "normal setting expansion." When additional water is brought into contact with the setting material, an increased expansion is observed. This latter expansion has been termed "hygroscopic expansion." It is the purpose of this paper to present the results of a series of tests which support an explanation for this phenomenon.

There have been many investigations into the nature of hygroscopic setting expansion, and several theories have been proposed. On the basis of considerable evidence, the consensus appears to favor the hypothesis that the actual mechanics of hygroscopic setting expansion is the same as for normal setting expansion—namely, a manifestation of the outward growth of gypsum crystals. The only difference, then, between hygroscopic setting expansion and normal setting expansion would be the addition of water to the setting mass; and it would seem indicated that the key to the explanation of this phenomenon must lie in the role played by this additionally supplied water. It is noteworthy that almost all previous experimentation has been concerned with the extent of hygroscopic setting expansion under varying conditions of composition, manipulation, and setting environment; and, although several authors have speculated on the role of the additionally supplied water, there is no direct evidence in support of these speculations. Since the emphasis of this investigation was directed toward the additionally supplied water, a review of these previous explanations is presented, followed by some comments based on presently available information.

Degni[1]—additional water is imbibed and causes a swelling of the gypsum gel formed by the reaction of water and hemihydrate.

Jorgensen[2]—additional water prevents the drying-up of intercrystalline spaces which would otherwise cause precipitation of gypsum crystals in areas not producing expansion.

Ryge and Fairhurst[3]—additional water (or other liquids) is absorbed by the setting investment because of a reduction in true volume and increases the surface-film thickness on inert particles and gypsum crystals, thereby forcing them apart.

Lyon, Dickson, and Schoonover[4]—additional water permits further hydration of calcium sulfate during a period when the investment is in a semisolid state.

Fusayama[5]—additional water (or other liquids) is pulled in to fill spaces produced

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by initial expansion. This liquid supports the space in a manner like a filler and helps the crystal ends grow farther. Thus the presence of additional fillers of this nature facilitates the expansion and makes it earlier and greater.

Degni's theory[1] does not explain the increased expansion that occurs when alcohol or oils are the added liquids, as demonstrated by Ryge and Fairhurst.[3] On the basis of gel characteristics, the addition of alcohol should cause a shrinkage.[6] In addition, the existence of a gel is controversial, with evidence against.[7]

Jorgensen's theory[2] specifies the simple requirement of water-filled intercrystalline spaces. Before the "loss of gloss," the intercrystalline spaces are filled in the case of both normal and hygroscopic setting conditions, yet the expansion at this point is greater for the hygroscopic setting conditions.[5] Furthermore, the increased expansion resulting from the addition of liquids other than water is not satisfactorily explained by this theory.

Ryge's theory[3] requires a penetration of added liquid throughout the mass, which is not supported by die tests, which indicated just surface penetration of the added liquid.[8]

Lyon's theory[4] specifies further hydration of calcium sulfate, which is not supported by X-ray diffraction data.[3]

Fusayama's theory[5] contends that the additional water is pulled into the spaces produced by the hygroscopic expansion and yet produces further hygroscopic expansion. It is difficult to visualize the additional water as a cause as well as an effect. The explanation of the additional water supporting intercrystalline spaces in the manner of a filler, thereby helping crystal growth, is vague.

As a consequence of these speculative and controversial explanations, this investigation was undertaken with the specific purpose of determining the cause of hygroscopic expansion by determining the role of the additionally supplied water.

PROPOSED EXPLANATION

It is proposed that the mechanism of the expansion, under both normal setting conditions and hygroscopic setting conditions, is a result of the same phenomenon—namely, the outward growth of gypsum crystals.

It is proposed that a given gypsum product under a given set of manipulative and environmental conditions has a given potential or maximum capacity for expansion which is realized under full hygroscopic setting conditions.

It is proposed that this maximum capacity is not realized under normal setting conditions because of an expansion-restraining force resulting from the surface tension of the mix liquid.

As a basis for this theory, a principle of a combined solid-phase–liquid-phase system is presented in Figure 1. Two solid particles are shown suspended in a liquid medium. The liquid volume is caused to diminish in proceeding from left to right, and, since wetting forces maintain solid and liquid contact, the particles are drawn together until they contact. Further loss of liquid volume increases the peripheral surface of the liquid phase, which brings surface-tension forces into play, and the particles are forced together. The full force of surface tension in compressing the particles together is realized just before the liquid film breaks or becomes discontinuous.

During the normal setting of gypsum products, a similar situation prevails. When

the hemihydrate of calcium sulfate ($CaSO_4 \cdot \frac{1}{2}H_2O$) is mixed with water, there occurs a progressive dissolution of the hemihydrate and a growth from solution of the dihydrate of calcium sulfate ($CaSO_4 \cdot 2H_2O$ — gypsum). The setting environment consists of a decreasing solid-phase volume of hemihydrate powder particles, an increasing solid-phase volume of gypsum crystals, and a decreasing liquid-phase volume of calcium and sulfate ions in solution with water. Although the final volume ($CaSO_4 \cdot 2H_2O$) is less than the original volume ($CaSO_4 \cdot \frac{1}{2}H_2O + \frac{3}{2}H_2O$), an apparent volume expansion (setting expansion) results, which is attributed to the outward growth of gypsum crystals. Thus we have the similar situation of solid phases present in a decreasing liquid-phase volume.

Under normal setting conditions the growing gypsum crystals meet the diminishing peripheral surface of the liquid phase soon after mixing. Wetting forces maintain contact between liquid and solid, and further decreases in liquid-phase volume result in increases in the surface area of the liquid surface, and surface-tension forces compress the solid-phase constituents together. Outward expansion is inhibited by these compressive forces, and crystal growth is forced to continue in areas of lesser resistance—namely, the intercrystalline spaces. Voids subsequently form within the mass which fill

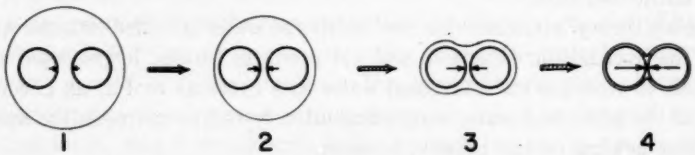

FIG. 1.—Schematic sketch demonstrating the compression of two solid phases by forces due to the surface tension of a surrounding liquid phase.

with water vapor. As these voids form, still greater forces are exerted by the large increase in the surface area of the liquid-vapor interfaces. It would appear likely that crystal growth would avoid extending or increasing the liquid-phase surface wherever possible, since each extension would be met with greater oppositional force.

If additional water is supplied before this point is reached, however, outward expansion continues uninhibitedly.* Supplying additional water simply increases the volume in which crystals may grow outwardly before the peripheral surface of the liquid phase is extended to bring surface-tension forces into play. Unlimited water supplied will allow the expansion to realize its full potential, whereas no water supplied will be the most restrictive of expansion. Water supplied between these limits will be manifested in unrestricted expansion until the peripheral surface of the liquid phase is extended, whereupon surface-tension forces will then inhibit outward crystal growth.

During expansion, the liquid phase of the setting gypsum material is continuous and adjusts to the addition of water so that increased volume is available at all points and not just in the area of the added water. The added water, therefore, need not and does not penetrate any deeper than the surface of the setting gypsum material.

It is significant to recognize that compositional factors (size, nature and amount of

* The term "uninhibitedly" here and elsewhere in the text refers to effects produced by surface-tension forces only. Intercrystalline forces, which develop as the reaction progresses, are also inhibiting but are excluded from this term for the convenience of this explanation.

silica in casting investment, accelerators, and retarders) and manipulative factors (water/powder ratio and conditions of spatulation) have not been mentioned, since these factors affect the inherent potential of a gypsum material to expand which is demonstrated in a similar manner under both hygroscopic and normal conditions.

This proposed theory is shown schematically in Figure 2, where the solid and liquid phases are shown and the progress of the reaction and expansion under normal and hygroscopic conditions are differentiated. The dimensional change curves for these stages of setting are also shown. *H* and *N* label the hygroscopic and normal setting curves, respectively. Although an attempt has been made to demonstrate additional points in the setting procedure, the schematic, rather than realistic, nature of this diagram should be kept in mind. Symmetry has been utilized as a means of drawing convenience.

In step *I* the initial mix is shown, where the outer circle represents the peripheral boundary of the liquid mix and the three round particles, which are enclosed, represent

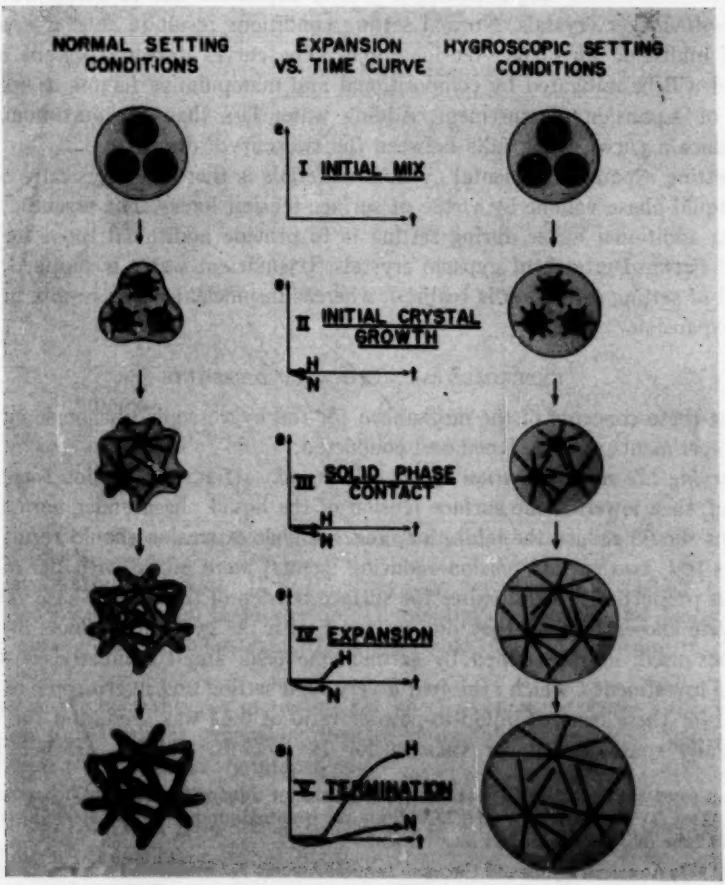

FIG. 2.—Schematic sketch of setting expansion under normal setting conditions and hygroscopic setting conditions.

undissolved particles of the hemihydrate of calcium sulfate. In step *II* initial crystal growth is represented by the radial lines extending from the surface of the hemihydrate particles.* At this point, in the case of normal setting, the utilization of water in the reaction results in the solid phases being drawn together, and a contraction is recorded. In the case of hygroscopic expansion, water lost in the reaction is compensated for by additionally supplied water, and no dimensional change is manifest. In step *III* the solid phases make contact, and no further peripheral contraction can occur. In step *IV* expansion commences. In the case of hygroscopic setting, the additionally supplied water continues to compensate for water lost in the reaction and provides space for outward crystal growth. In the case of normal setting, however, the loss of water produces an extension of the peripheral boundary of the water phase, with surface-tension forces compressing the solid phases together and inhibiting outward growth. Crystal growth and precipitation concentrate in the intercrystalline spaces, while voids form and fill with water vapor. In step *V* the expansion ends, with hygroscopic conditions demonstrating a greater expansion due to uninhibited crystal growth with longer crystals. Normal setting conditions result in shorter crystals because of inhibition by surface-tension forces. The curves drawn represent a general form and will be mitigated by compositional and manipulative factors as well as the method of expansion measurement. Adding water less than the maximum amount will produce a curve which falls between the two curves drawn.

The setting expansion of dental gypsum materials is therefore physically contained by the liquid-phase volume by virtue of surface-tension forces. The essential effect of providing additional water during setting is to provide additional room for the uninhibited outward growth of gypsum crystals. If sufficient water is supplied, the full potential of setting expansion is realized, whereas insufficient water results in reduced setting expansion.

EXPERIMENTAL METHODS AND RESULTS

To test these concepts of the mechanism for the hygroscopic phenomenon, the following experiments were designed and conducted:

A. *Varying the surface tension of the mix liquid.*—If surface-tension forces inhibit expansion, then lowering the surface tension of the liquid phase under normal setting conditions should reduce the inhibition, and a higher expansion should result.

In this test, two surface-tension–reducing agents† were mixed with the mix water in various proportions so as to alter the surface tension of the liquid phase. These two agents were chosen because they did not react with the gypsum products in the concentrations used, as determined by setting-time tests and colorimetric observation. A casting investment,‡ which exhibited a very high setting and hygroscopic expansion, was used for these tests. A liquid-to-powder ratio of 0.32 was used, and the mix was mechanically spatulated under vacuum for 20 seconds.§ The mixed material was

* There is some question as to the nature of the nucleus for this crystal growth. Photographs shown by Skinner (ref. 9) and Jorgensen (ref. 2) indicate the hemihydrate particle, whereas Luster (ref. 8) suggests that the dihydrate forms its own nucleus.

† Aerosol OT, American Cyanamid Co., and Tergitol Anionic 7, Carbide and Carbon Chemicals Co.

‡ Hygrotrol, Whip Mix Corp.

§ Vac-U-Vestor, Whip Mix Corp.

placed in a flexible rubber inlay ring.* A fine wire was welded to a stainless-steel-mesh platform, and this was placed on top of the investment. No water was added for this test, and the axial expansion was measured by following the movement of the end of the wire with a micrometer slide cathetometer.†

In Table 1 are shown the proportions used for these two surface-tension–reducing agents and the effect on the surface tension of the mix liquid. It is interesting to note that it took a greater percentage of Tergitol to achieve the same reduction in surface tension than in the case for Aerosol. In Figure 3 is shown the relationship between

TABLE 1

EFFECT OF ADDING SURFACE-TENSION–REDUCING AGENTS TO MIX WATER OF DENTAL CASTING INVESTMENT

	Surface Tension (Dynes/Cm)	Gillmore Initial Set Time (Min.)	Linear Expansion (Per Cent)*
	Aerosol OT Concentration (Per Cent)		
0	72	9.0	0.56
0.0014	50	9.0	0.54
0.0049	42	10.0	0.52
0.0170	33	10.0	0.57
0.0570	29	9.5	0.75
0.1000	26	9.0	0.98
	Tergitol Anionic 7 Concentration (Per Cent)		
0	72	9.0	0.56
0.0010	55	9.0	0.52
0.0100	44	9.0	0.58
0.1000	36	9.0	0.61
0.2000	31	9.0	0.84
0.5000	28	10.0	1.07
1.0000	26	9.5	1.18

* Average of three runs. Standard deviation no greater than 0.05 per cent.

surface tension and expansion. It is apparent that a reduction in surface tension is accompanied by an increase in expansion. The points for Tergitol and Aerosol follow the same curve, which demonstrates the unique role of surface tension in this phenomenon.

B. *Alternately reducing and increasing the effect of surface-tension forces.*—If surface-tension forces inhibit expansion, then bringing these forces into play suddenly should inhibit the expansion simultaneously.

In this test, hygroscopic water available to the setting investment was introduced through a capillary tube mounted in the bottom of a ring filled with investment. The same conditions prevailed as in test A except that water was used in the mix and the

* Water Added Technic Ring, Ransom & Randolph Co.

† Gaertner Scientific Corp., 0.001-mm. instrument accuracy.

expansion was measured automatically by actuating a cantilever-beam transducer utilizing SR-4 wire strain gauges. As a result of straining the beam, the wire strain gauges change resistance, and, when connected in a Wheatstone bridge circuit, a voltage change can be derived. This is relayed to an X-Y-T recorder,* where the expansion is automatically plotted versus time. A schematic drawing of this test setup is shown in Figure 4. In Figure 5 is shown a picture of the recording where the hygroscopic water supply has been alternately turned off and on for several cycles. This results in surface-tension forces being brought into play (water off) and out of play (water on), respectively. Since the liquid phase is rapidly decreasing, as soon as the additional water supply is halted, the growing crystals are right at the peripheral boundary of the liquid phase. As can be seen, the effect on expansion is immediate. When the water is turned off, the expansion ceases immediately and is resumed only when the water is turned back on. There is no perceivable time lapse between turning

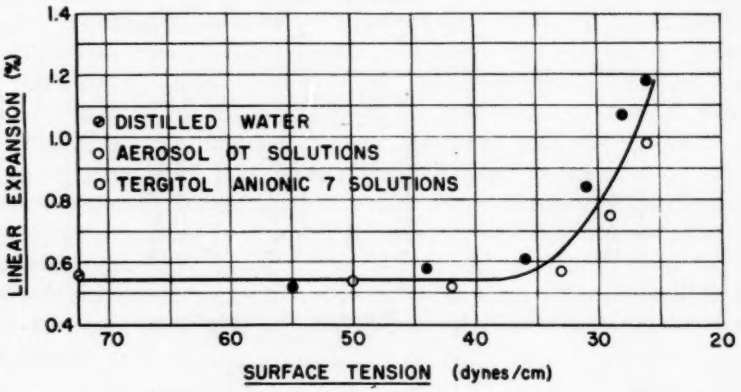

FIG. 3.—Relationship of setting expansion to the surface tension of the mix liquid

the valve and the alteration of expansion as determined by qualitative observation (½ second or less). The absence of any perceivable time lag between action and reaction specifically points to the presence of a force mechanism in this phenomenon. At times, as soon as the water is turned off, an actual drop in expansion can be observed, demonstrating the presence of a compressive force. When the water is allowed to remain off, the plateau is extended, and, when the liquid film breaks, expansion continues, but at a much reduced rate.

C. *The amount of expansion as related to the amount of water absorbed.*—If the added water provides additional room for expansion before the peripheral surface of the liquid phase is reached by the growing crystals, then the volume expansion (V_E) produced should be related to the volume of the added water (V_A). Since there is a true volumetric contraction (V_C) accompanying the conversion of hemihydrate to dihydrate, the added water must compensate for this volume loss as well. The following relationship should then hold as long as sufficient hygroscopic water is available:

$$V_A = V_E + V_C .$$

* F. L. Moseley Co.

Several tests were run to check the validity of this equation. First, V_C was determined for the materials and conditions of this study, using the method outlined by Mahler and Asgarzadeh.[10]

V_E was determined by adding the axial expansion (as measured by the strain-gauge transducer) to twice the lateral expansion as measured by an orthodontic band of stainless steel and measuring 0.005 inch in thickness, which was shaped in the form of a circle to conform to the outside of the rubber ring. The ends of the band were overlapped, and a measuring microscope was focused on two cross-marks scratched near each end of the band. The measurement was made between these marks and represented the circumferential expansion of the ring. The lateral expansion was computed from this figure. A few tests demonstrated that the lateral measurement at the center of the ring represented the average lateral expansion for the entire ring.

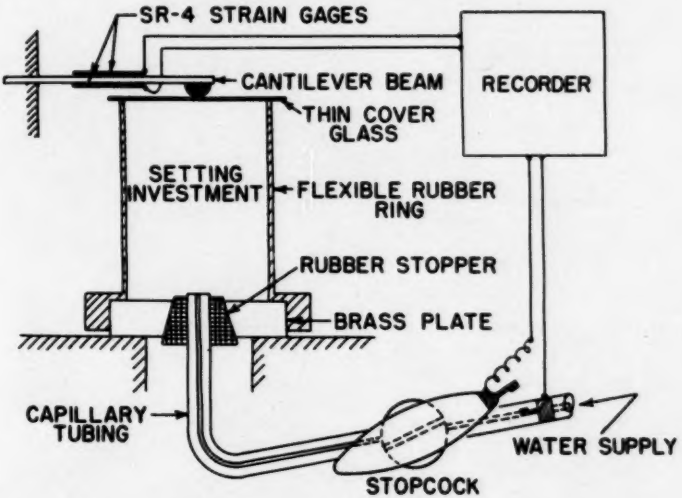

FIG. 4.—Schematic sketch of the apparatus used to record simultaneously the setting expansion and the on-off operation of the added hygroscopic water.

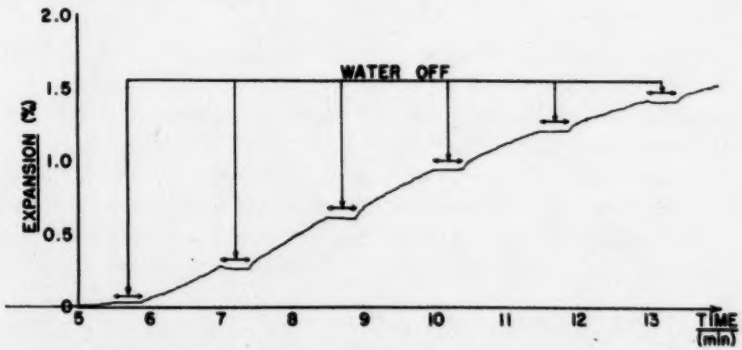

FIG. 5.—Actual recording made of the influence of surface-tension forces (added water alternately turned off and on) on setting expansion.

V_A was measured by recording the drop of the water column in the calibrated capillary tube connected to the bottom of the investment plug.

All these measurements were recorded simultaneously during the setting period. In Table 2 are shown the results of adding unlimited water to plaster, hydrocal, densite, and casting investment. It can be observed that, within the limits of experimental error, the volume of water added (V_A) is equal to the sum of the volume setting expansion (V_E) due to crystal growth and the volume contraction (V_C) due to the reaction. These values were taken at 60 minutes from the start of mix.

In Table 3 are shown results of tests conducted with casting investment where the

TABLE 2

COMPARISON OF V_A AND $V_E + V_C$ FOR VARIOUS GYPSUM MATERIALS—UNLIMITED WATER ADDITION

Material	No. Runs	V_E+V_C (Cc.)	V_A* (Cc.)
Plaster	5	0.59±0.01	0.54±0.09†
Hydrocal	5	0.59±0.01	0.64±0.05
Densite	5	0.71±0.03	0.68±0.01
Investment	5	1.43±0.05	1.49±0.03

* Volumes measured between 5 and 15 minutes from start of mix.
† Arithmetic mean ± standard deviation.

TABLE 3

COMPARISON OF V_A AND $V_E + V_C$ FOR CASTING INVESTMENT—DIFFERENT WATER ADDITIONS

No. Runs	V_A (Cc.)	V_E+V_C Cc.)*
5	0.50	0.51±0.03†
5	1.00	1.05±0.09
5	1.50	1.52±0.07
12 (unlimited)	2.37±0.18	2.21±0.26

* Volume measured between start of mix and point at which water was shut off.
† Arithmetic mean ± standard deviation.

amount of water added was restricted. The values for V_A, V_E, and V_C were taken only up to the point where the water supply had been shut off, since V_E and V_C would continue after this point but V_A could not. Once again, the results demonstrate the validity of the equation $V_A = V_E + V_C$.

In Figure 6 is a curve of V_A versus $V_E + V_C$ for unlimited water added to casting investment. The curve is for the entire setting procedure from 3 minutes to 60 minutes after mix. Here, again, the relationship of $V_A = V_E + V_C$ is established. This series of tests validates the concept that hygroscopic water provides additional space for expansion.

D. *Liquids added other than water.*—An interesting aspect of the hygroscopic phenomenon was pointed out by Ryge and Fairhurst[3] when liquids other than water were added to the setting investment. The expansion for the case of these other liquids was

shown to be greater than the normal setting expansion but not so great as when water was the added liquid. It might appear that if the added liquid provides room for additional expansion, then there should be no difference in adding different liquids. However, if an interface is formed between the added liquid and the liquid phase of the mix which cannot be penetrated by the growing gypsum crystals, then interfacial-tension forces would inhibit expansion in the same manner as surface-tension forces do in the case of normal setting.

In this test, various liquids were added to the top of the setting casting investment, and the axial expansion was determined by the wire and cathetometer method. One cubic centimeter of each liquid was added at 3 minutes from the start of mix, and the expansion was determined at the 60-minute mark. The data are shown in Table 4.

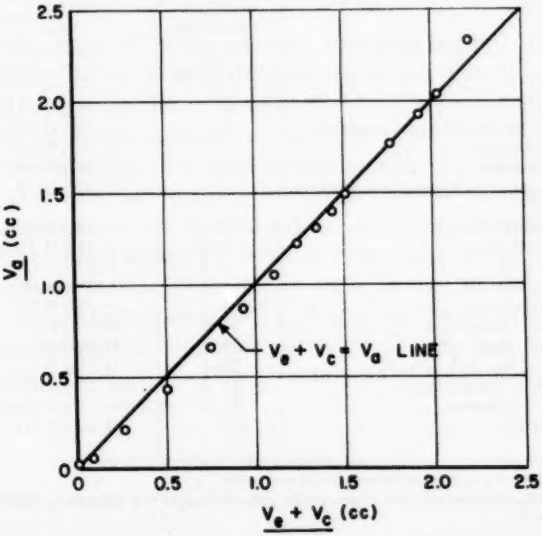

FIG. 6.—Relationship of the volume of hygroscopic water absorbed to the apparent volumetric setting expansion plus the true volume loss due to the conversion of hemihydrate to dihydrate.

It can be seen that those liquids which do not form interfaces and are miscible in water result in the same expansion as that demonstrated by the addition of water, within limits of experimental accuracy.* Those liquids that do form interfaces, however, result in much less expansion, which is of an order approximating normal setting expansion. Furthermore, the amount of expansion appears to be related to the interfacial tension between the added liquid and water. A plot of interfacial tension versus expansion for liquids forming interfaces is shown in Figure 7. The effect was not so pronounced as in the case where the surface tension of the entire liquid phase was altered (Aerosol and Tergitol additions), since only part of the outer surface had been subject to surface-tension reduction rather than the entire liquid phase. However, an

* These results do not corroborate Ryge's results for adding other liquids (see ref. 3). A possible reason for this discrepancy may be that, in Ryge's method of measuring, only the expansion on the top surface of the mass was measured, and this measurement could be easily influenced by the presence of the added liquid at this point.

increase in expansion can be seen with a reduction in interfacial tension of the top surface of the setting mass.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to provide an explanation for the phenomenon of the hygroscopic setting expansion of gypsum materials. The tests conducted were specifically directed to determine the role of the additionally supplied water. The following points were established.

TABLE 4

SETTING EXPANSION OF CASTING INVESTMENT WHEN ADDING 1 CC. OF VARIOUS LIQUIDS

Liquid Added	Interfacial Tension* (Dynes/Cm)	Linear Expansion (Per Cent)
Water	0	2.18±0.21†
Saturated solution of gypsum	0	2.29±0.07
Ethyl alcohol	0	2.29±0.10
P-Dioxane	0	2.26±0.15
0.1 per cent Aerosol OT solution	0	2.30±0.08
Ethylene glycol	0.27	1.94±0.10
Glycerine	0.44	1.34±0.19
3 in 1 oil	8.4	0.85±0.03
Richfield No. 1 oil	9.2	0.74±0.01
Richfield No. 2 oil	11.2	0.75±0.03
Mobil furnace oil	11.6	0.79±0.02
Mobil diesel oil	18.8	0.79±0.05
Mobil No. 615 lube oil	29.4	0.71±0.03
Carbon tetrachloride	41.2	0.75±0.04
Mobil kerosene	52.0	0.73±0.03
Mercury	375	0.62±0.06

* Interfacial tension between liquid and water.

† Each expansion value represents the arithmetic mean of 5 test runs ± standard deviation.

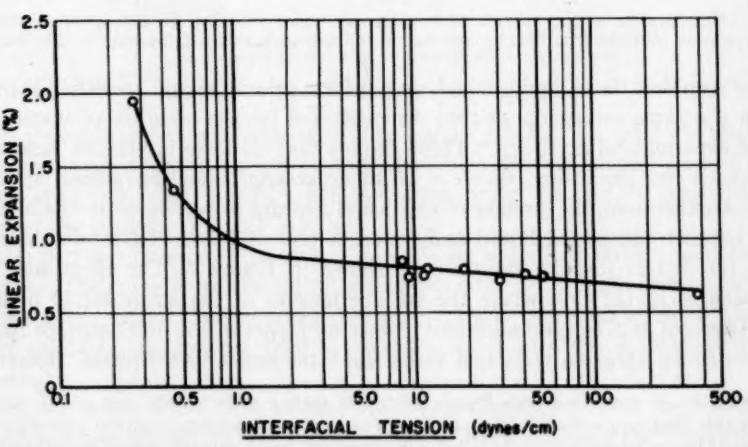

FIG. 7.—Relationship of setting expansion to the interfacial tension between the mix liquid and liquids added other than water.

A. Setting expansion is related to the surface tension of the mix liquid.

B. Surface-tension forces, when brought into play, inhibit setting expansion, and the converse is true.

C. The volume of the additionally supplied water is equal to the resulting volumetric hygroscopic setting expansion (after subtracting that part of the added water which compensates for the volume loss accompanying the conversion of $CaSO_4 \cdot \frac{1}{2}H_2O + \frac{3}{2}H_2O$ to $CaSO_4 \cdot 2H_2O$).

D. Adding liquids other than water does not change the magnitude of the hygroscopic setting expansion, provided that these liquids are miscible or do not form interfaces with water. Immiscible liquids inhibit the setting expansion in accordance with the values for their interfacial tension with water.

These results substantiate the following explanation for the phenomenon of hygroscopic expansion:

The setting expansion of dental gypsum materials is physically contained in its liquid-phase volume by virtue of surface-tension forces. The full expansion potential is realized when sufficient water or certain other liquids are brought into contact with the setting material (full hygroscopic conditions). This allows for an outward growth of gypsum crystals into the additional volume provided by these added liquids, with no buildup of surface-tension forces. If no liquid is added (normal setting conditions) or if insufficient liquid is added (partial hygroscopic conditions) or if a liquid forming an interface with water is added, all of which allow the growing crystals to reach and extend the peripheral surface of the liquid phase before the full expansion potential has been realized, surface-tension forces of the mix liquid inhibit outward crystal growth, and the expansion is reduced.

Thus hygroscopic setting expansion is simply setting expansion uninhibited by the action of surface-tension forces.

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An Epidemiological Study on Dental Caries among High-School Students in Athens, Greece

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In the search for an understanding of the factors having a bearing on dental caries immunity or susceptibility, the application of the epidemiological methodology as an effective research tool has been demonstrated from studies conducted during the last two decades or so.[1-3] The present paper gives an account of an epidemiological study on caries which was carried out recently among Greek school children.

The purposes of the study were the following: (*a*) to investigate the influence of World War II and postwar conditions on caries prevalence and (*b*) to determine the dental status and dental needs of a group of children by employing standard epidemiological procedures and indexes (D.M.F. teeth index and its components), so that comparisons with similar groups can be made. No studies of such nature and extent have been reported previously for Greece.

MATERIALS AND METHODS

The dental examinations were carried out among children of two public high schools in Athens—one for boys and one for girls. (Public high schools in Athens are separate for boys and girls.) These schools were included on the basis of co-operation on the part of the principals and adequacy of facilities available for conducting the examinations. Attendance in high schools of Athens is not limited only to children residing in the vicinity of a school. Children living in different parts of the city can and do attend schools which are not located in their immediate neighborhoods. No attempt was made to determine whether or not the children included in the investigation were born and reared in Athens.

The subjects of this study were children twelve, thirteen, and fourteen years of age. The fourteen-year-old children were born during the last year of World War II. Those thirteen and twelve years of age were born during the first two years following the end of hostilities.

The examinations were performed by mouth mirror and explorer. The mouth was illuminated by a dental spotlight. The teeth were dried with a chip blower prior to the examination. A dental head-rest attached to an ordinary chair was used for the examinations. All dental examinations were done by the author—a fact which adds uniformity to the material collected. The findings were recorded on an appropriate dental record by an assistant.

The caries experience of the subjects was recorded in terms of D.M.F. teeth. Teeth

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This investigation was conducted by the author in 1959 during a sabbatical leave from the University of Oregon Dental School.

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lost from causes other than caries were not taken into consideration. A tooth both carious and filled was counted only once in the total D.M.F. teeth index. In computing the components of the index, such a tooth was counted twice; once in the "D" category and once in the "F" category.

The dental examinations of the subjects were limited to the teeth of one side of the mouth only, that is, right upper and lower quadrants. This procedure was adopted on the basis of the well-established phenomenon of the bilateral occurrence of dental caries observed in population groups. Recent studies among university students and school children have demonstrated that limiting the observations to the teeth of one side of the mouth only resulted in caries attack rates which, when they are multiplied by 2, give the attack rates for the entire mouth with a high degree of accuracy.[4-6] In the present study, when no evidence of caries attack was observed on the teeth of the right side of the mouth, the left side was also examined before a child was classified as truly caries-free.

TABLE 1

NUMBER OF CHILDREN EXAMINED, D.M.F. TEETH PER CHILD, AND PER CENT CARIES-FREE BY AGE GROUPS

ITEM SPECIFIED	AGE IN YEARS (LAST BIRTHDAY)		
	12	13	14
Number examined....	150	150	142
D.M.F. teeth*.....	3.76	3.72	3.70
Per cent caries-free...	16.0	16.7	19.0

* Rates for the D.M.F. teeth index represent the whole mouth.

RESULTS

The age distribution of the children included in the study, as well as their caries experience rates, is presented in Table 1. Altogether, 442 subjects were examined in the three age groups combined. The number of boys and girls for each age group was about the same.

The average number of D.M.F. teeth per child is expressed for the whole mouth. This was done by multiplying the figures obtained from the examination of the right side of the mouth by 2. The data presented in Table 1 regarding the caries experience of the subjects are of considerable interest. As can be seen, the D.M.F. rates are practically the same for each of the three age groups. On the other hand, there are differences in each age group in the percentage of children who are caries-free. These differences, however, are in the opposite direction of what would be normally expected. That is, as the age of the group advances, the percentage of children caries-free shows an increase instead of a decrease. The lowest percentage of caries-free children was found among those born two years after the end of World War II (twelve years old) and the highest among subjects born during the last year of hostilities (fourteen years old).

The components comprising the total caries experience of the children (D.M.F.) are presented in Table 2, by specified age groups. The figures for each component are expressed again for the whole mouth.

The information given in this table demonstrates the state of dental care of the children examined, indicated by the amount of dental services received in contrast to the unmet dental needs of the population group. A comparison between the average number per child of teeth which need to be filled and teeth extracted because of extensive caries versus teeth which have been filled shows that between 86 and 95 per cent of the dental needs of the children have not been met.

DISCUSSION

The school children of Athens included in the present study demonstrate a remarkable resistance to caries attack. This is indicated from the data presented in Table 3,

TABLE 2

COMPONENTS OF D.M.F. TEETH PER CHILD BY AGE GROUPS

COMPONENTS*	AGE IN YEARS (LAST BIRTHDAY)		
	12	13	14
Decayed	3.26	3.08	2.92
Missing	0.30	0.24	0.34
Filled	0.24	0.42	0.48

* Rates for the components represent the whole mouth.

TABLE 3

COMPARISON OF CARIES EXPERIENCE BETWEEN CHILDREN 12–14 YEARS OF AGE IN ATHENS, GREECE, AND FOUR LOCALITIES IN UNITED STATES

(Rates Expressed in Terms of D.M.F. Teeth per Child)

LOCALITY	AGE IN YEARS		
	12	13	14
Athens, Greece	3.76	3.72	3.70
Kewanee, Illinois	2.83	3.48	4.00
Grand Rapids, Michigan	3.87	5.05	6.78
Evanston, Illinois	5.63	6.76	7.93
Providence, Rhode Island	7.07	8.43	9.55

which compare their caries rates with those reported among children from different areas of this country. In general, the D.M.F. teeth rates of the Greek children are rather similar to those observed among subjects who had lived continuously in Kewanee, Illinois, and had consumed water containing naturally 0.9 ppm of fluorine.[7] Furthermore, the percentage of children in Athens and Kewanee who are caries-free, for the three age groups combined, is also similar—17.2 and 17.9 per cent, respectively. The children who were continuous residents of Grand Rapids, Michigan, and consumed during the last ten years of their lives water containing about 1.0 ppm of fluorine show caries rates which are higher in comparison with those of the Greek

children.[8] The D.M.F. teeth rates of children living in Evanston, Illinois, who were examined by Dean and associates in 1941 prior to fluoridation of the public water supply, and Providence, Rhode Island, are considerably higher than those observed among the Greek subjects.[7, 9]

The high degree of resistance to caries attack found among the Athenian children of the present study cannot be attributed to the beneficial effects of fluorine. Samples from the public water supply of Athens were found to contain only traces of fluorine.[10-11]

The influence of World War II and postwar conditions on the prevalence of caries among children from different European countries has been reported in various studies.[6, 12] In general, it has been observed that caries activity decreased during the war years, and then it started increasing again in the postwar period. The explanation for this phenomenon has been attributed by different investigators to one of the following three factors: (*a*) the consumption of sugar and sugar products decreased materially during the war years; (*b*) the diet consumed in the period of war exerted a favorable nutritional influence during the developmental period of the teeth, which resulted in an increased resistance to caries attack; and (*c*) a combination of the above two points of view.

For an explanation of the remarkable degree of immunity to caries attack noted among the children of the present study, the possible influence of conditions prevailing in Greece during the World War II and postwar years should be commented upon. The second World War greatly affected the health of the Greek people. During the famine period (1941–42) thousands of persons died from starvation. In a survey conducted during the spring of 1945 it was found that children were even shorter and lighter than those examined in another survey about two years earlier.[13] Following the end of the war in Europe in 1945, Greece was in a state of near-collapse as a result of widespread destruction. Furthermore, a civil war broke out upon liberation, which greatly aggravated the task of reconstruction. As a result of these conditions, the rehabilitation of the country and the return of the population to normalcy, particularly from the point of view of diet, was delayed in comparison with other European countries.

In an effort to interpret the dental caries pattern observed among the Athenian children, the possible influence of certain nutritional factors deserves primary consideration. From recent studies among European children, it has been suggested that changes in the amount of sugar consumed during and after the war was the primary factor responsible for the observed changes in caries prevalence rates.[6]

During the period of the occupation of Greece, sugar was almost unobtainable. Following the liberation of the country, sugar became available again, and in 1948 it was estimated that about 9 kg. per person per year were consumed.[10] In 1958 sugar consumption stood at about 18 kg. per person per year.[14] In general, the per capita consumption of sugar in Greece appears to be less than that in other European countries and the United States. However, in interpreting the relation of sugar consumption to the caries experience, certain pertinent facts must be kept in mind. On the basis of personal knowledge of and familiarity with conditions prevailing in Greece, it is believed that interpretations based on the amount of sugar consumed per person for the country as a whole may lead to erroneous conclusions. In general,

there is an extreme disparity in income, availability, and variety of foodstuffs, and standards of living between persons residing in Athens and the more than half the population of Greece who live in small villages and towns. A variety of sugar products is available in the homes and in the shops of Athens, which suggests a considerable intake of sugar. In view of the above, it is felt that no reliable conclusions can be reached regarding the amount of sugar consumed by the children in Athens on the basis of the per capita consumption for the country as a whole.

In the present study, the teeth of the fourteen-year-old children, which developed partly during the war but mostly in the postwar period, showed the highest resistance to caries. For the children born the first two years following the end of hostilities (thirteen and twelve years of age) resistance to caries attack decreased progressively. The teeth of all the children erupted some years after the war at a time when sugar and sugar products were readily available. However, although their teeth were exposed to the same oral environment, the older children displayed more resistance to caries than did the younger ones. Apparently, some factor operating during the period of tooth development may have influenced the resistance of teeth to caries. It seems that the presence of this beneficial factor decreased gradually from the last year of the war to the first few postwar years.

The influence of diet during the developmental period of teeth on caries susceptibility has been investigated by Sognnaes on rodents.[15] In one group of animals a stock diet (natural ration) was fed to the mothers during pregnancy and lactation and then to the offspring for the duration of the experimental period. In the second group a cariogenic (purified) diet was given to the mothers during the periods of pregnancy and lactation, and the same diet was fed to the young during the experimental period. He found that the first group of animals showed a very low caries incidence in comparison with that of the second group. The lower caries experience observed in the first group of animals was attributed to a nutritional influence operating during the developmental period of the teeth.

From some observations available on dental conditions of school children in Athens and also from the results of the present study, it appears that for the last 23 years their susceptibility to caries has remained largely unaltered. In an examination conducted by Krikos in 1936 among ten-year-old children attending one school in Athens, it was found that 14 per cent of them were caries-free.[10] The teeth of these children were formed during the prewar years and also erupted in the mouth in the same period. In a second survey conducted by Krikos among children of the same age group and in the same school of Athens ten years later (1946), it was reported that 16 per cent of the subjects were caries-free.[10] The teeth of the children were formed partly before and partly during the second World War and erupted in the mouth during a period when sugar was almost unavailable. A third survey was made by Coumoulos in 1947 among children six years of age attending public schools in Athens.[10] The results showed that 22 per cent of them were free of caries in their primary teeth. The teeth of these children were formed entirely during the years of occupation, when sugar was scarce, and erupted in the oral cavity in the same period.

In reviewing the similarity in the degree of caries susceptibility among the Athenian children during the last 23 years, one cannot avoid the conclusion that some factor

or combination of factors of nutritional origin and acting during the period of tooth development may be responsible for bringing about an increased resistance to caries. The nature of the factor(s) is not understood at the present time. However, the findings of recent studies tend to suggest that certain trace elements may be responsible for decreasing or increasing the susceptibility of teeth to caries attack.[16-18] Furthermore, it may be of interest to mention that the consumption of fresh fruits and vegetables in Greece has been reported to be among the highest in Europe.[19]

The possible association between sunshine and caries susceptibility, which has been pointed out in the past, deserves consideration in the present study. Recent epidemiological studies in different geographic areas of Oregon have also indicated the existence of an inverse relationship between the amount of sunshine available and caries prevalence rates.[20] Children living continuously east of the Cascade Mountain Range, where more hours of sunshine are available, showed low caries rates in comparison with those residing west of the mountain range with fewer hours of sunshine available. In view of this, the amount of sunshine available in Athens was calculated for the last 7 years from data supplied by the Meteorological Institute of the National Observatory of Athens.[21] It was found that about 68 per cent of the possible hours of sunshine was available per year. In contrast, Portland, Oregon, which is located west of the Cascades, had only about 44 per cent of the possible sunshine available for the same 7-year period.[22]

As a result of favorable climatic conditions in Athens, children spend considerable time in outdoor activities, including swimming and sun bathing at the nearby beaches of the city. Exposure to sunshine results in the formation of vitamin D through the action of the biologically effective component of the ultraviolet radiation of the sun on the provitamin present under the skin. There are indications from the literature that an adequate intake of this vitamin during the developmental period of the teeth may increase their resistance to caries attack, all other factors being equal.[23] However, since the biologically effective component of the sunlight, which lies at wave lengths of 3,150 A and shorter of the solar spectrum, may be influenced by atmospheric pollution from fog, haze, etc., the construction of an apparatus for measuring this component of solar radiation will greatly enhance our knowledge in this area.

From the data presented in Table 2, it is evident that the dental care of the children included in this study has been grossly neglected. Almost all their caries experience is comprised of teeth with open cavities. Undoubtedly, a number of factors may be responsible for this situation, such as the economic conditions of families and lack of appreciation of the value of dental care. The availability of dentists does not seem to be a factor, since about half the dentists in Greece are practicing in Athens. It is believed that a decisive step toward increasing interest in and appreciation of dentistry for children, on the part of both the dentists and the public, will be accomplished by the establishment of a chair in pedodontia at the University of Athens Dental School and also at the newly created dental school at the University of Salonika.

SUMMARY

An epidemiological study on dental caries was conducted among school children twelve to fourteen years of age in Athens, Greece. The fourteen-year-old children were

born during the last year of the second World War in Europe, whereas those thirteen and twelve years of age were born during the first two years following the end of hostilities.

The children of the three age groups showed a marked resistance to caries attack. The older children, however, displayed higher immunity to caries than did the younger ones.

The fluoride content of the public water supply of Athens was not the factor responsible for the high degree of resistance to caries. The amount of sugar consumed after the eruption of the teeth did not seem to have influenced the caries pattern observed among the subjects.

The findings of the study indicate that some nutritional factor operating during the period of tooth development may have been responsible for the marked resistance to caries. Data from previous caries surveys in Athens tend to support this view.

The dental care of the children was found to be grossly neglected, and a measure is proposed for improving this situation.

Appreciation is expressed to Mr. Panos Patras, principal of the Second High School for Boys, and Mr. George Stefopoulos, principal of the Ninth High School for Girls, for their kind co-operation in this study. The assistance of Dr. Athanasios Papademetriou in supplying the dental instruments used in this study is gratefully acknowledged.

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Sclerotic Age Changes in Root Dentin of Human Teeth as Observed by Optical, Electron, and X-Ray Microscopy

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A preliminary survey of age changes in human teeth by Nalbandian,[1, 2] utilizing the multifactorial point system proposed by Gustafson,[3] suggested that the relative transparency of root dentin—generally known as "sclerosis"—might be among the more reliable dental aging characteristics, presumably least affected by the superimposed pathology to which the crown portion of teeth is prone. It seemed worthwhile, therefore, to examine the nature of the age changes in the root dentin more closely.

In the present study structural alterations associated with sclerotic root dentin have been observed by means of optical and electron microscopy as well as by high-resolution microradiography. While similar techniques have been employed individually as applied to studies on crown dentin, there does not appear to be any previous study comparing the combined results of these several approaches in the study of typical sclerotic age changes in the root dentin of human teeth.

The classical studies of W. D. Miller,[4] Beust,[5–7] and Bodecker and Lefkowitz,[8, 9] demonstrated alterations in the optical appearance of ground sections of dentin as well as a decrease in permeability to dyes coincident with aging. These changes could be the result of intratubular calcification, which would inhibit the diffusion of dyes and cause the refractive index of the tubules to approach that of the intertubular dentin, thus modifying the relative transparency.

Recently a number of microradiographic investigations of crown dentin[10–16] have revealed areas of considerably greater X-ray absorption in relation to the dentinal tubules than in the general dentin matrix, suggesting deposition of highly concentrated minerals. For example, J. Miller[10] demonstrated a highly mineralized zone around the odontoblastic processes in microradiographs of dentin from unerupted teeth. Similar observations were made by Blake[11] in sound teeth from eleven- to thirteen-year-olds. Bergmann and Engfeldt[12] studied by microradiography the transparent dentin under superficial caries. Their material showed, in addition to hypercalcified zones around many tubules, other tubules which appeared to be completely obliterated by high-density material. The following year Röckert[13] reported complete tubular obliteration in the crown dentin of young and old *non-carious* teeth, stating that there was a greater tendency toward tubular obliteration with increasing age.

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Bradford[14, 15] has studied the structure and "maturation" of dentin by optical microscopy of ground sections and sections of decalcified material, noting various stages in a progression of tubular "maturation," culminating in complete tubular obliteration.

The most conclusive demonstration of a hypercalcified peritubular zone was recently made by Frank[16] in electron-microscopic studies of undecalcified sections of crown dentin from unerupted third molars.

It appears that the hypercalcified peritubular zone is a normal structure in dentin, with the possible exception of areas near the predentin, where microradiography indicates its absence.[17] In an unpublished microradiographic study of crown dentin performed in our laboratory, the peritubular zone was observed in *young non-carious* teeth, but not near the pulpal border. However, uncertainty exists as to whether tubular *obliteration* is an aging phenomenon or is related entirely to pathology and physiological wear and tear. That obliteration may be complete has not been clearly demonstrated. It must be borne in mind that optical microscopy and microradiography do not afford enough resolution to visualize great detail within dentinal tubules, especially in thick ground sections subject to the added problem of refraction artifact. Refraction in ground sections of dentin can produce the effect of ring formation in and around tubules, and for this reason some authors[18–20] attributed the translucency around the odontoblastic process to optical artifact.

While newer tools have been used to study normal and pathological *crown* dentin, the present report deals with an investigation of *root* dentin, where the irritation of odontoblastic processes by attrition, abrasion, caries, and other extraneous injury—wear and tear—would have less direct influence on the structural differences between the dentin of young and that of old individuals.

MATERIALS AND METHODS

Optical microscopy.—Specimens examined by reflected and transmitted light included, in part, thick tooth slabs for general orientation and, in part, ground sections and decalcified sections prepared by standard methods described by Sognnaes.[21]

Microradiography.—Small pieces of root dentin, $1 \times 1 \times 2$ mm., were cut from three distinct zones—opaque, transparent, and intermediate—utilyzing eight adult teeth selected from a large number because of their sharply delineated apical transparent dentin. In order to obtain cross-sectioned tubules, the isolated pieces were ground and polished alternatingly on the pulpal and cemental sides. Specimens of a thickness from 30 to 60 μ were exposed for about 8 minutes on spectroscopic film* using a contact microradiographic apparatus† adjusted to 4 kv. and 4 mA. For direct comparison with the microradiographs, the sections were next mounted and optical micrographs made. Pieces of root dentin were obtained from similar sites on fully erupted teeth of a younger age group (eleven- to fourteen-year-old) and treated as just described.

Electron microscopy.—From areas of adult dentin, exhibiting the typical transparent and opaque zones, minute cubes (less than 1 cu. mm.) were fixed for 24 hours in 80 per cent alcohol and imbedded in 10 per cent methyl- in *n*-butyl methacrylate, with great care in orientation of the specimens. Sections were cut without decalcifi-

* Kodak, type 649 GH.

† Philips.

cation with a diamond knife mounted on a microtome,* collected on water, and picked up on grids covered with a carbon film. Some sections were floated on versine (to remove inorganic matter) prior to examination in the electron microscope. Electron micrographs were obtained with an electron microscope† at 50 kv. with a 50-μ objective aperture. Original magnifications on the plates were 2,000–13,000×, subsequently enlarged photographically two to seven times. Selected area diffraction patterns were made of material within tubules as well as of intertubular matrix.

RESULTS

Coincident with aging, root dentin becomes transparent, giving rise to a glasslike appearance in transmitted light. This change commences at the root tip, creeping toward the crown. By examining longitudinal tooth slabs in the dry state (Fig. 1), we

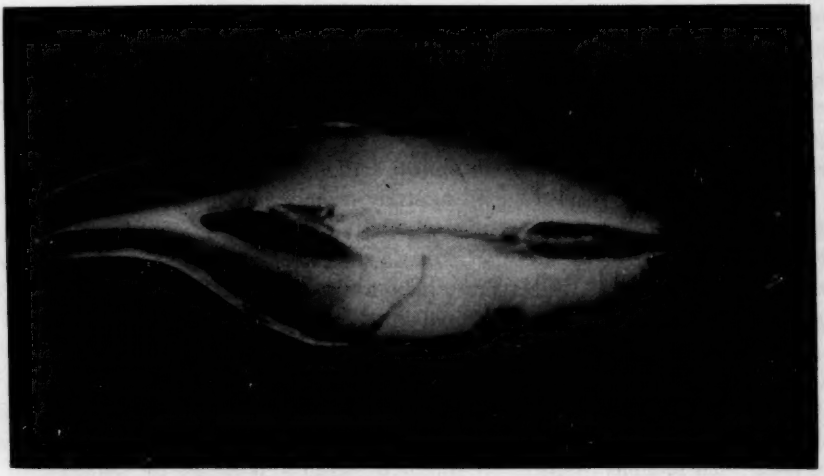

FIG. 1.—Longitudinal central slab of an undecalcified adult tooth (0.5 mm. thick) photographed by transmitted light shows the transition between opaque (*white*) and transparent root dentin (Mag. ×3.)

have selected areas representing transitions between these optically different varieties of dentin, exploring the nature of tubular mineralization with more refined tools.

Microradiographic observations by the technique described indicate that the distribution of transparency in root dentin of old teeth is correlated with the degree of tubular sclerosis. Root dentin from teeth of young and old is compared in Figure 2.

In young root-tip dentin all tubules are easily visualized in cross-cut ground section, there being a large, dark central zone containing the odontoblastic process, surrounded by a translucent ring (Fig. 2, *A*). In the corresponding microradiograph of the same area (Fig. 2, *A1*) the site of the odontoblastic process is indicated by a uniform central radiolucency, surrounded by a narrow zone of increased microdensity in the area of the translucent zone.

In that part of the root dentin of old teeth which is still opaque in reflected light,

* Sorvall-Porter-Blum.

† RCA, type EMU 3B.

the tubular structure generally appears similar to that in young dentin, both optically and microradiographically (Fig. 2, *B* and *B1*). However, a few tubules may appear to be filled partially or completely with radiodense material, especially near the junction of transparent dentin. Figure 2, *B* and *B1*, shows three tubules (*see arrows*) which have undergone various stages of intratubular calcification, representing an overlap of transparency and opaqueness.

In transparent dentin the tubules are generally difficult to visualize in unstained ground sections, because of a lack of natural contrast in the tissue. But the micro-

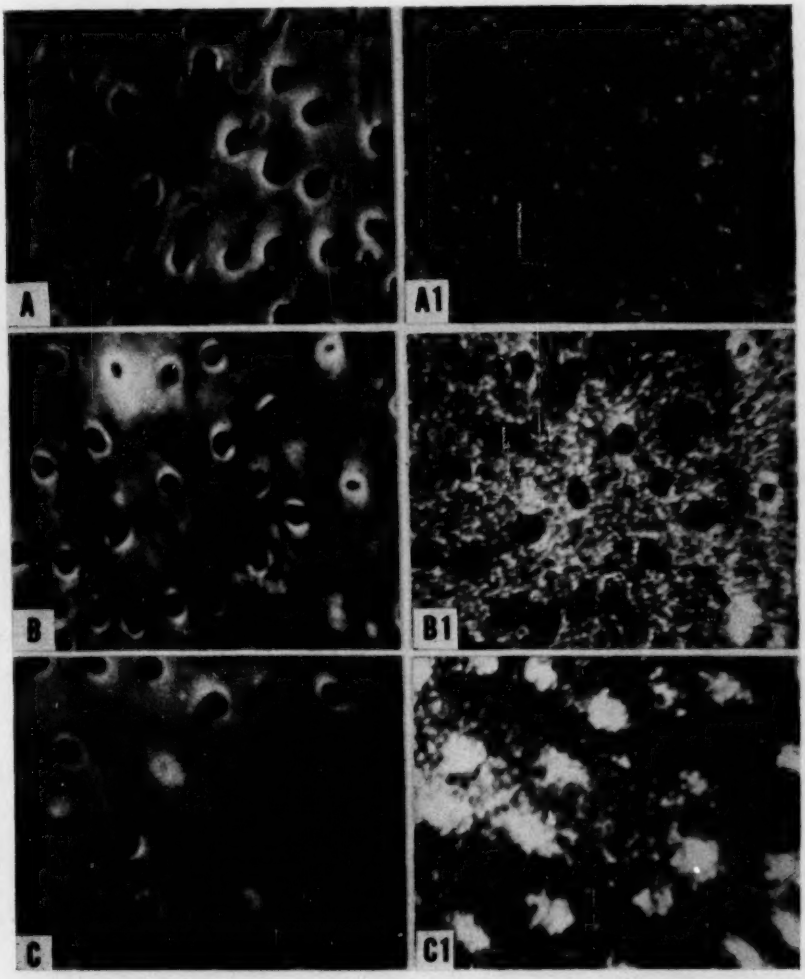

FIG. 2.—Optical micrographs (*left*) and corresponding microradiographs (*right*) of identical areas from three sites: *A* and *A1*—typical opaque root dentin in the apex of a fully formed young tooth from a thirteen-year-old individual; *B* and *B1*—opaque root dentin from an adult tooth from a sixty-four-year-old individual; *C* and *C1*—transparent root dentin in the same tooth apical to the site shown in *B*. (Mag. ×1,800.)

radiographs indicate that a very high degree of intratubular mineralization has occurred in this type of sclerotic dentin, as compared in Figure 2, *C*, a photomicrograph of a ground section of transparent dentin, and Fig. 2, *C1*, a microradiograph of the identical area. These illustrations show a number of partially calcified tubules with enough tissue contrast to localize corresponding areas in the micrographs, when viewed by the two methods, respectively.

Electron micrographs of ultra-thin sections of undecalcified dentin from opaque zones of the root exhibited masses of calcified collagen fibers coursing in the intertubular areas, mainly in directions perpendicular to the long axis of the tubules—that is to say, in planes parallel to the plane of section (Fig. 3, *A*). The contents of the tubules were of low electron density and poorly defined (Figs. 3, *A*, and 4, *A*). The general appearance of a section cut perpendicular to the long axis of the tubules was of a sieve, i.e., highly electron-dense material with fairly regular holes (about 2 μ in diameter), separated from one another by a distance of about 4 μ. In contrast to this picture, low-power micrographs of sclerotic dentin gave the appearance of a solid sheet of electron-dense material without holes (Fig. 3, *B*). It was only by careful observation on the electron-microscopic screen that obliterated tubules could be seen. These were areas about 1.5 μ in diameter showing a series of parallel breaks perpendicular to the knife marks (Figs. 3, *B*, and 4, *B*).

The material in the tubules was also different in texture from that constituting the

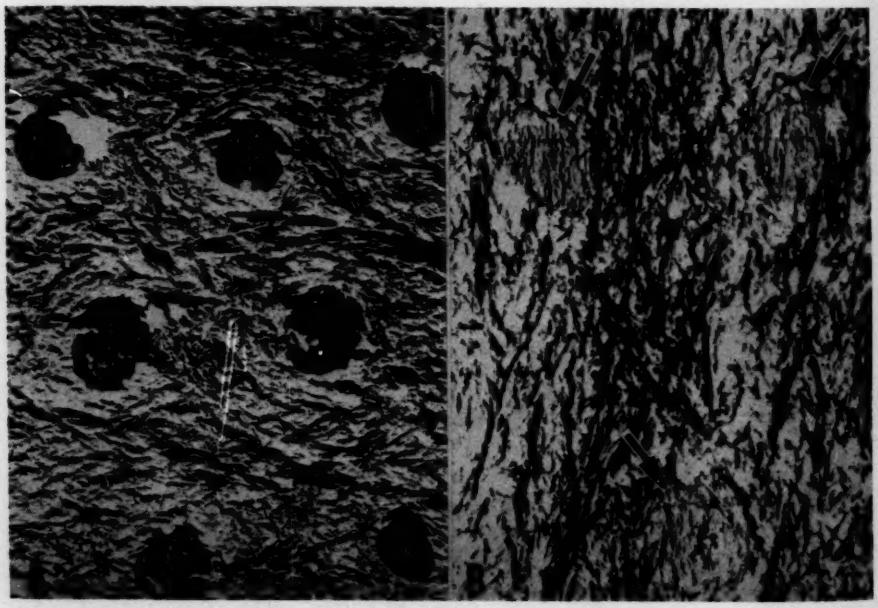

FIG. 3.—Electron micrographs of undecalcified sections of root dentin, cut by a diamond knife. (Photographs are reversed, so that white represents the most highly mineralized material.) *A*—opaque root dentin, showing the regular array of dentinal tubules (*dark holes*) cut in cross-section. *B*—transparent root dentin, showing the cross-cut tubules (*arrows*) filled with extremely electron-dense material. (Mag. ×4,600.)

intertubular matrix. In Figure 4, *B*, one can see crystallites oriented mostly parallel to the plane of section in areas outside the obliterated tubule, whereas the area within (*MT*) presents a stippled appearance. In the sectioned tubules of opaque dentin there is a narrow border of calcified material exhibiting breaks and stippled texture very much like that of obliterated tubules of transparent dentin (compare Fig. 4, *A* and *B*). This border, probably corresponding to the peritubular matrix, is not uniform in thickness but represents the appearance of two half-moons on either side of the tubular contents. The thin parts of the border lie on opposite sides of the tubule, determining an axis always perpendicular to the direction of breaks in the border.

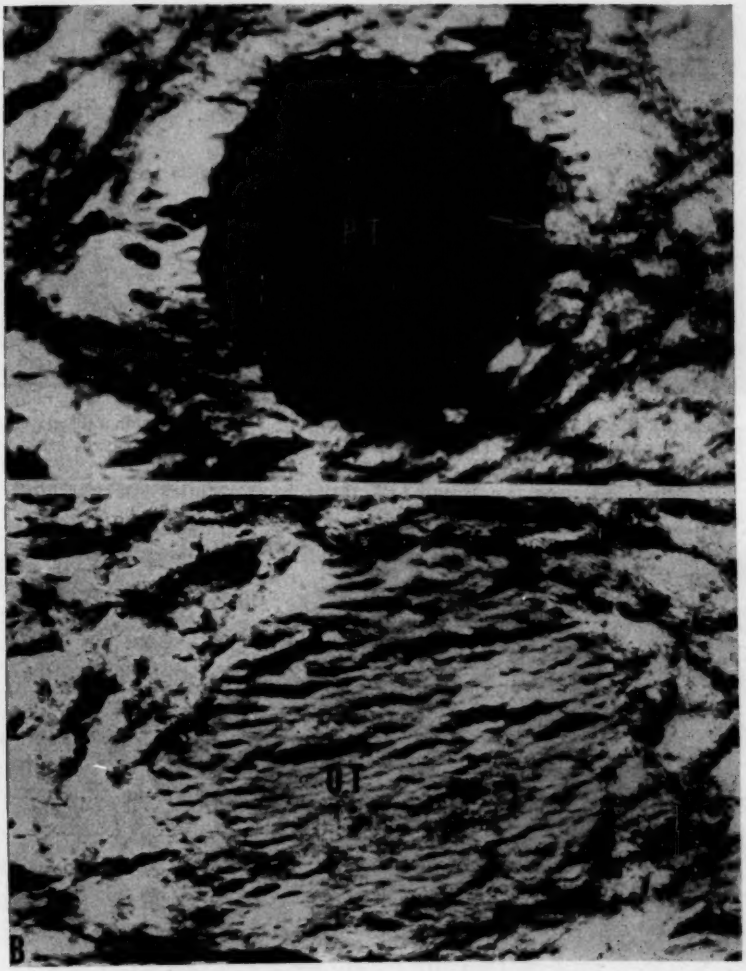

FIG. 4.—Electron micrographs of undecalcified sections of opaque (*A, above*) and sclerotic (*B, below*) root dentin. *A*—patent dentinal tubule (*PT*) in opaque dentin, showing, between arrows, a ring of calcified peritubular matrix; *B*—occluded dentinal tubule (*OT*) in sclerotic dentin. Note similar texture and "breaks" in peritubular matrix and in material within sclerotic tubule. (Both. mag. ×35,000.)

Selected area diffraction patterns made within filled tubules of sclerotic dentin (Fig. 5, *left*) and of areas outside the tubule (Fig. 5, *right*) were identical, suggesting a similar crystal lattice. But the organic template may not be identical, because sections of dentin decalcified in versine and subsequently examined in the electron microscope showed that the material remaining within patent tubules (Fig. 6, *A*) differs from that remaining in the sclerosed tubules (Fig. 6, *B*), which failed to reveal distinct fibrillar structures.

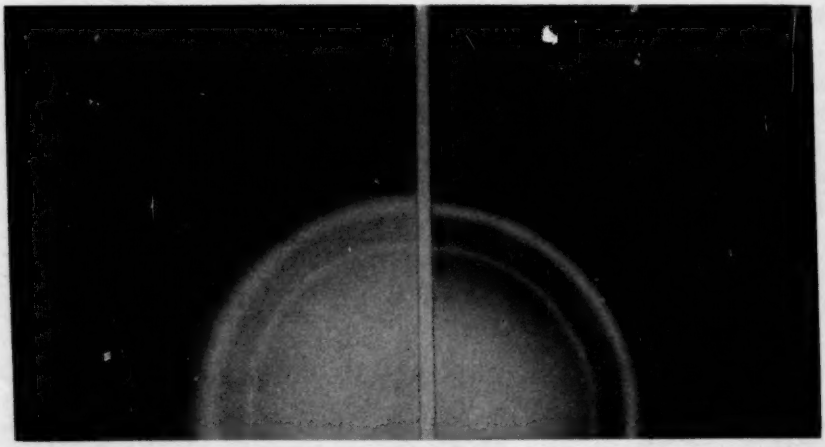

FIG. 5.—Selected area diffraction patterns of material within a sclerotic mineralized tubule (*left*) and of intertubular matrix (*right*). The ring spacings are identical and compatible with an apatite lattice.

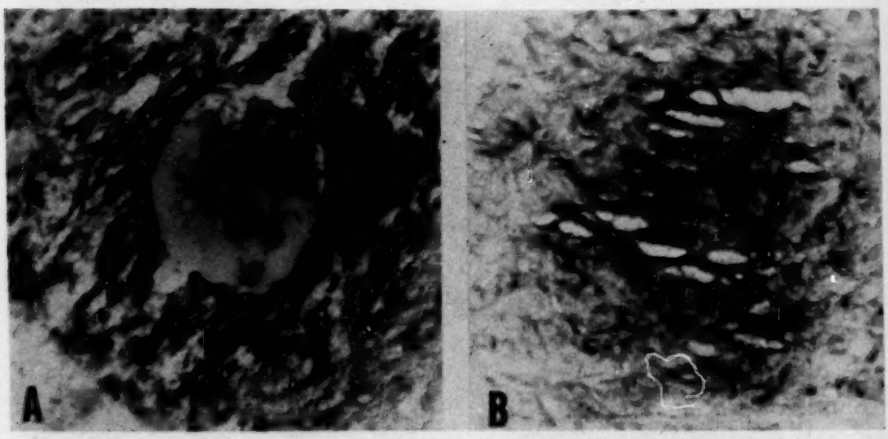

FIG. 6.—Electron micrographs of organic remnants of root dentin from an adult tooth which was decalcified after sectioning without removal of methacrylate imbedding material. *A*—a patent tubule; *B*—an occluded tubule (equivalent to Figs. 3, *B*, and 4, *B*). Note that the occluded tubule retained a moderately electron-dense organic material after decalcification. (Mag. ×16,000.)

DISCUSSION

Consistent with the earlier findings of J. Miller,[4] Röckert,[13] and Blake[11] (related to crown dentin of young or unerupted teeth without caries), our microradiographs and optical observations show that young root dentin also has a peritubular hypercalcified area, though it may be narrow and require careful examination to be detected in microradiographs. In young root dentin the over-all diameter of the tubules is smaller than in crown dentin and is separated by a greater amount of intertubular dentin.

A striking change appears to occur in areas of transparency in old teeth. Microradiographs suggest a complete mineralization of tubular contents throughout the transparent area by material of a similarly high X-ray and electron density as that of the peritubular matrix. Nonetheless, some zones occupied by minute odontoblastic processes may be seen when focusing on the surface of the corresponding ground section.

The apparent discrepancy between the optical and radiographic views of transparent dentin (as illustrated in Fig. 2, *C* and *C1*) may be explained on the basis of tissue thickness. In other words, the X-ray picture represents a projection through a thickness of tissue, as a result of which any angulation of the tubules tends to cause an overlap to give the picture of complete obliteration. Microradiographs may therefore show complete obliteration before this is apparent from focusing on the upper surface of a ground section. In very transparent areas, ground sections optically examined show, in fact, that a majority of the tubules are devoid of demonstrable odontoblastic processes.

The narrow zone of highly calcified material found in electron micrographs around the patent tubules and exhibiting breaks and stippled texture may be interpreted to correspond to the peritubular matrix seen in the microradiographs of similar material. In the fully sclerotic tubules, only one line of junction is seen, i.e., between the highly calcified substance and the intertubular dentin. This is easily visualized because the different texture and consistency have led to compression artifacts, especially in ultrathin sections (see Fig. 4, *B*).

That the breaks seen in the electron micrographs of sclerosed tubules are compression artifacts is indicated by the observation that they always appear perpendicular to knife scratches. The texture and consistency, as well as the type of breaks in the sclerotic material, appear similar to those in the walls of patent tubules of opaque dentin. The thin portions of the half-moons of peritubular dentin were always on opposite sides of the patent tubules and on an axis perpendicular to the breaks.

In all probability, the peritubular matrix is more uniform in thickness than the electron micrographs indicate. Additional sheaths, as suggested by Bradford's scheme of dentin maturation,[14] have not been seen in this study. If these exist, failure to see them may be due to the type of fixation or to masking by the very electron-dense mineral. Neither were any sheaths seen after decalcification, however; yet it should be emphasized that the material was not treated with electron-dense dyes (e.g., osmic acid and phosphotungstic acid). It appears that the intratubular calcification is continuous with what would have been referred to as peritubular matrix before maturation had

occurred (compare Fig. 4, *A* and *B*). These observations suggest a continuous inward growth of the peritubular matrix.

Our electron diffraction patterns, which showed an apatite crystalline component in the mineralized tubules, add weight to the above suggestion, in that the peritubular matrix of crown dentin is also apatite.[16]

In electron-microscopic studies of carefully decalcified coronal dentin, Frank[22] has demonstrated a fibrillar material in a zone interpreted to be the peritubular matrix. The organic material in this zone is extremely fragile, however, and difficult to preserve. For this reason, sections of decalcified material may shed light on the existence of the peritubular zone. We have no definite clue regarding the nature of the organic matrix material in the sclerotic tubules. Decalcification of our ultra-thin sections on the grids showed a residual organic material in sclerotic tubules. Further study with electron staining may yield information on whether or not there exists a fibrillar matrix. It would be surprising if none were present, inasmuch as calcification generally occurs in relation to a fibrillar framework. Yet it is difficult to explain fibrogenesis in tubules distant from fiber-forming cells. Perhaps the basic ingredients for calcification are supplied by the odontoblast cell extensions.

In interpreting mechanisms of tubular sclerosis, it should be kept in mind that different processes may be responsible at different sites, e.g., under caries, attrition, abrasion, erosion, and the apical dentin sclerosis with which this paper deals. The optical appearance of sclerotic areas may differ and has been referred to as "opaque and transparent sclerosis."[5] This may even be related to secondary pathologic and physiologic conditions other than mere chronologic and biologic age. In one area, at least—namely, the transparency under caries—a different mechanism may be involved than in apical sclerosis, inasmuch as Frank[17] has described an abnormal type of crystalline structure in the tubular deposits found adjacent to caries.

SUMMARY

1. An investigation of the microstructural differences between opaque and transparent (sclerotic) root dentin was carried out by light microscopy, microradiography, and electron microscopy.

2. By light microscopy and microradiography root dentin from young individuals has the same appearance as opaque root dentin from mature adults. The tubules have a large radiolucent center (odontoblastic process) surrounded by a translucent and highly radiopaque zone (peritubular matrix).

3. In apical sclerotic dentin, the transparency of which increases and spreads crownward as a tooth matures, the tubules are generally difficult to visualize when viewed in the light microscope, most of them being very narrow, with inconspicuous odontoblastic processes. In microradiographs the tubules appear to become virtually completely obliterated by a highly radiopaque substance.

4. Electron micrographs correlate well with the microradiographic findings. The opaque dentin, cut in sections transverse to the tubules, shows an electron-dense material interrupted by circular areas of low electron density corresponding to the patent tubules. In contrast, transparent dentin contains no such circular areas but is of relatively homogeneous electron density throughout. Sclerosed tubules appear to have a different texture and consistency from that of the intertubular dentin.

5. Electron diffraction patterns of areas within the obliterated tubules were identical with those of intertubular dentin, indicating that crystals deposited within sclerotic tubules in the root are of the same type as those found in the intertubular dentin.

6. Sections decalcified by treatment in versine showed a relatively homogeneous organic matrix remaining within mineralized tubules.

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Measurement of the Physiological Mobility of Individual Teeth in an Axial Direction

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Forces such as those produced during the mastication of food cause the tooth to move in its socket. This movement is resisted by the resiliency of the tissues surrounding the tooth, particularly those comprising the periodontal membrane.

Although lateral movements have been measured by Muhlemann[1-8] and others, only a few devices have been described that are capable of measuring the small physiological axial tooth movements. Zwirner[9, 10] described a method utilizing the movable plates of a condenser, one attached to the tooth to be measured and the other to the substance in which the jaws of a rat were imbedded. Picton[11] described a clinical method of measuring axial tooth movement by means of a resistance-strain gauge. One end of the gauge was attached to a single tooth and the other through a spring to the adjacent teeth.

Axial movement has been studied because direct measurement of tension or compression of tissues can be made as the tooth can be moved bodily in this direction without tilting, so that all points on the tooth move the same amount and in the same direction. The points of application of the measurement devices are, therefore, not critical.

MATERIALS AND METHODS

Design of the present machine.—The present machine utilizes the rectilinear transducer (Extensometer micrometer). This is essentially a center-tapped coil with movable core, widely used in industry to transform movement into a voltage change. The two coils of the transducer act as two legs of an inductive-type bridge, the other two legs of which are in the external circuit. When the core is moved, the permeance of the coils varies. These instruments are extremely sensitive and accurate, and over a considerable range of movement the change in voltage is linear. Transducers are powered by translator instruments that supply a constant amplitude of variable-frequency voltage, amplify the transducer output, and rectify it into a d.c. voltage which is shown on a meter or passed on to a continuous recorder.

The machine consists of two systems; one follows the movement of the tooth, and the other measures an applied force. Both systems utilize a transducer. The mechanical system which follows tooth movement consists of a bit held in the molar and premolar regions on both sides of the mouth and on which are compound impressions of the upper and lower teeth. A hollow rod projects from the corner of the mouth, and the measurement device is attached to this by universal clamps.

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The measurement device (Fig. 1) consists of a subminiature transducer from which extend two probes—a measurement probe from the movable and floating core and a reference probe from the coil housing. The ends of these probes are 3 mm. apart and rest on the incisal or occlusal surfaces of two adjacent teeth. A fine screw adjustment raises and lowers the reference probe to take up any discrepancy between the height of the two adjacent teeth without disturbing the zero position of the measurement probe. The measurement probe and core are suspended by fine parallelogram springs, which allow the movements of the tooth to be followed and the core to move freely through the coils of the transducer while giving rigidity in other directions. A worm-gear adjustment allows the probes to be brought into contact with the teeth with

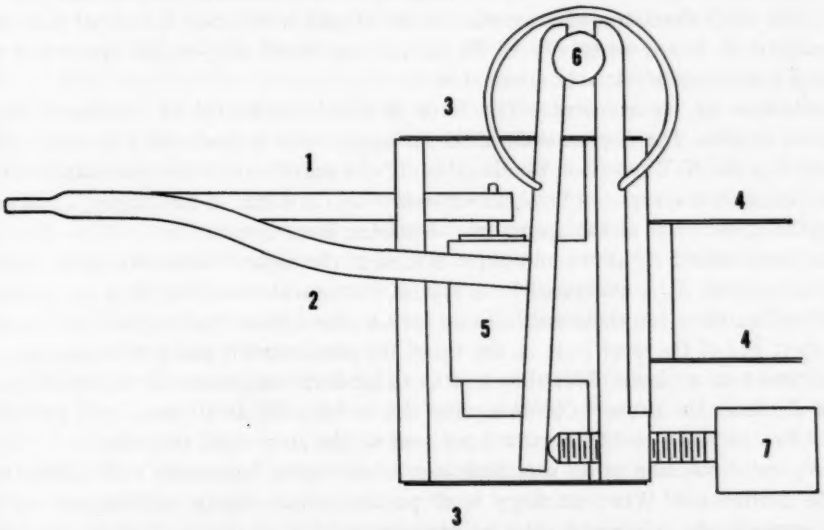

FIG. 1.—The movement-measuring device. (*1*) The measurement probe; (*2*) the reference probe; (*3–3*) the parallelogram springs carrying the measurement probe and transducer core; (*4–4*) the parallelogram suspension springs carrying the device; (*5*) the transducer; (*6*) the reference probe adjustment; (*7*) the reference probe adjustment screw.

controlled force through a more rigid set of parallelogram springs, and the universal clamps make positioning of the probes possible. A fine three-way cable connects the transducer to the translator instrument.

The mechanical system for applying and measuring force is an entirely separate system. It consists of another slightly larger transducer mounted in a pen-type holder. The movable core is carried by a highly polished rod sliding in a Teflon bearing and projecting from the end of the holder. It is held at zero position by a coiled spring. On the end of the rod is a non-slip pad which is applied to the incisal edge of the tooth. Force is applied manually through the transducer holder and is conveyed to the sliding rod and floating core by means of the coiled spring. When force is applied to the tooth, the rod carrying the core depresses the spring by an amount commensurate with the force applied, and this is continuously measured by the transducer. A fine three-way cable connects this transducer to a second translator instrument. The move-

ment and force systems are therefore mechanically and electrically separate entities, combined only when the force is applied to the tooth being measured.

The output of the two systems can be read on the d.c. meters or passed to continuous recorders. Two types of recorders are used—an X-Y recorder which is a null-seeking servo-type plotter with two independent axes, and a continuous-balance servo strip-chart recorder. The X-Y recorder records force and movement; time is read on a stop watch. The strip-chart recorder records movement and time; force is read on the d.c. meter.

The X-Y recorder records on standard $8\frac{1}{2} \times 11$-inch graph paper. It has a sensitivity of 10 mv. per inch and a pen speed of 7.5 inches per second in each direction. It is provided with a continuously variable attenuator allowing easy calibration to scale. The strip-chart recorder records on an 11-inch-wide chart driven at 4 inches per minute. It has a sensitivity of 50 mv., a pen speed of $\frac{1}{4}$-second span, and an external continuously variable attenuator.

Calibration of the machine.—The force device is calibrated by loading it with standard weights. The displacement of the spring and core so produced is automatically recorded by the X-Y recorder. By alteration of the sensitivity of the translator instrument, full scale readings can be obtained from 8 to 1,500 gm. in five stages, 1,500 gm. being the upper limit of the spring in the present force device.

The device which measures movement is used at the highest sensitivity of the translator instrument. It is calibrated by a special instrument consisting of a micrometer gauge calibrated in ten-thousandths of an inch acting against the long end of a lever. The short end of the lever rests on the tip of the measurement probe when the device is positioned in a clamp. Five-thousandths of an inch movement of the micrometer gauge displaces the probe 0.001 mm., and this is recorded as 10 mm. ± 10 per cent on the X-Y recorder or 22.3 mm. ± 7 per cent on the strip-chart recorder.

The present machine varies very little over many weeks, but at any time calibration can be carried out. When accuracy is of particular importance, calibration can be made immediately before and after an experiment.

Position of the movement-measuring device.—The movement-measuring device can be oriented approximately normal to the long axis of the upper incisor teeth by setting the probes at an angle of 70° to their labial surfaces.

When a tooth is deflected labially or lingually, it rotates about a point within its root. It is highest in relation to the other teeth when vertical and decreases in height as it becomes deflected. The angulation of the probes is adjusted so that labial and lingual movements give equal meter deflections, denoting an equal decrease in height in each direction. During this adjustment, only the contact pressure of 5 gm. or less is maintained on the tooth. However, when a tooth is depressed in its socket by an axial load in excess of 15 gm., it behaves as if it were slung by oblique fibers from each side, and, when deflected labially and lingually, it rises in its socket. The angulation of the probes is, therefore, further adjusted until labial and lingual movements of the tooth give equal meter deflections, denoting an equal increase in height in each direction. In this position the probe is considered to be in the functional long axis of the tooth.

Position of the force device.—The force device is held in the long axis of the tooth at right angles to the measurement probe and is applied to the tip of the tooth beside

it. When the movement-measuring device is correctly positioned, the angulation of the force device is not critical. The effect of alteration of the angle of the applied force on the axial movement of the tooth can be checked on the chart.

Stability of the reference points.—The stability of the movement-measuring device is of prime importance. The measurement probe is rigid and firmly attached to the transducer core. Bending of the measurement probe is negligible, for the only force that is transmitted through it is that sufficient for adequate contact with the tooth, which is between 1 and 5 gm. Movement of the core is essentially the same as that of the probe at the point of contact with the tooth. Registration of this movement depends upon the stability of the transducer coils.

The transducer coil housing is suspended from the apparatus held between the posterior teeth on both sides of the mouth. A reference probe extends from the transducer housing and rests on a tooth adjacent to the one being measured. This reference probe is held firmly on the tooth with a force of 100 gm. through the suspension springs.

Measurements show that relatively little axial tooth movement takes place with axial forces between 100 and 500 gm. One hundred grams of continuous pressure is always maintained on the reference tooth. The reference tooth is also seated by one or more applications of force of 500 gm. or more before measurements are taken. Stability of the reference tooth was determined by measurement of the relative heights of the measurement and reference teeth over a period of time. With 100-gm. contact pressure on the reference tooth and only instantaneous pressure of 0.5 gm. at 15-second intervals on the measurement tooth, no measurable change in the relative heights of the two teeth took place in 6 minutes.

Tooth movement was also measured from a single reference tooth and from a cap splint embracing six teeth. The difference between these measurements was negligible under 1 minute, but between 1 and 3 minutes greater movement was recorded from the splint. This suggests that, when measurements are made from a single reference tooth, the full movement of the measurement tooth may not be recorded, as the reference tooth itself may be slowly intruded. For short periods of time a single tooth offers considerable stability as the immediate anterior reference point. Where longer periods of measurement are required, however, the single reference tooth can be used after the axial displacement of this tooth with continuous force has reached its limit. This may take as long as 4 minutes but is often completed in under 1 minute.

Clinical tests.—Recordings of axial force and movement were made on a single upper incisor tooth of one individual during a period of 1 year, and the angulation of the probes and the settings of the machine remained undisturbed for as long as 6–8 weeks at a time. This served both as a study of the variations in tooth movement and as a test for the machine. Movements of the right and left central and lateral incisors of several individuals were also measured. Axial movement of the tooth was measured as follows: (1) by employing intermittent force, with alteration of the amount of force, the rate of application and removal, and the intervals of time between application; (2) by employing continuous forces of different but constant magnitude or of varying magnitude; (3) after the removal of continuous force; (4) during the retention of a smaller force subsequent to the removal of a larger force; and (5) under a variety of conditions, such as at different times of the day and night, following periods of sleep, guarding the teeth from all external forces, traction on the teeth,

the eating of different foods, the application of force for varying periods of time, change in posture, and after taking different drugs.

RESULTS

1. *Intermittent force.*—Immediately upon the application of axial force, the tooth moves (Figs. 2–4). The average movement of 500 recordings of one particular central incisor tooth was 0.0004 mm. with 1 gm., 0.012 mm. with 10 gm., 0.02 mm. with 100 gm., and 0.028 mm. with 1,000 gm. Similar movements have been observed on a limited number of other individuals. Initial intrusive tooth movement with small force

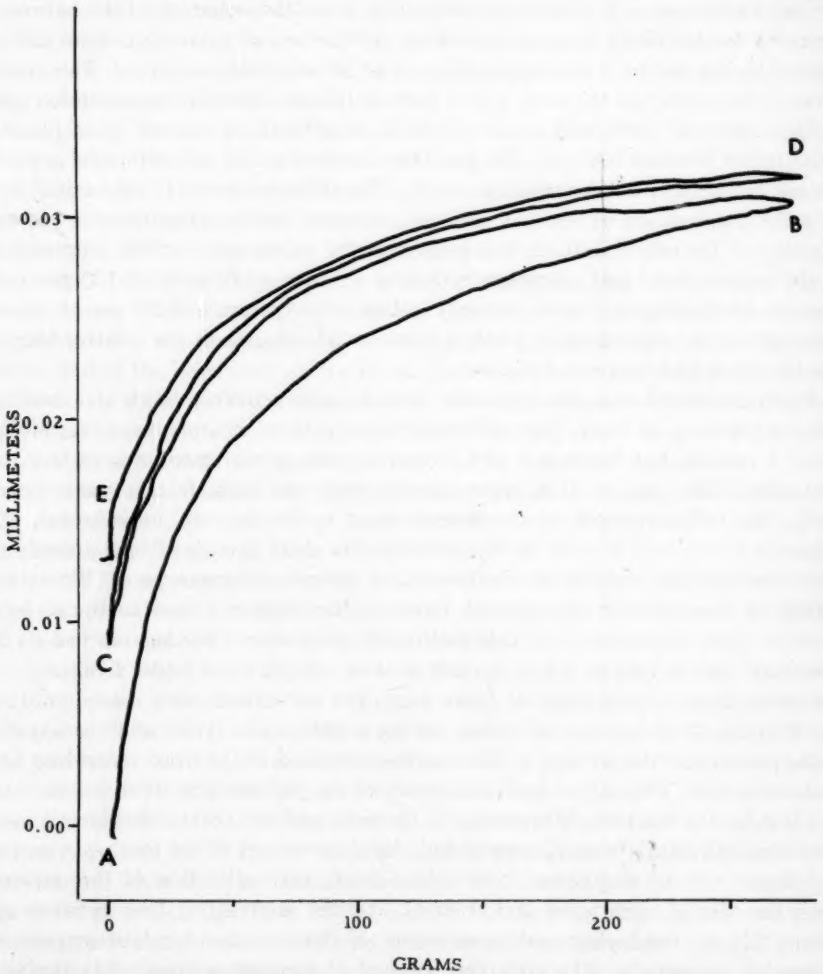

FIG. 2.—The axial force/movement curve of an upper incisor tooth on waking in the morning. (*A*) The initial position; (*B*) the position at peak force on the first application; (*C*) the return point on removal of force; (*D*) the position at peak force on the second application; (*E*) the return point after the second removal.

is relatively great, but progressive resistance to movement is encountered. The relationship of movement to force never becomes linear, but, so long as the force is applied gradually and evenly, the movement closely follows a logarithmic relationship with force. Upon repeated applications of force, this relationship is maintained.

Upon removal of force, the tooth recovers its original position in two phases. The first phase is a rapid recovery over part of the distance, and the second phase is a slow recovery to its original position. The rate of this slow recovery toward the original position diminishes progressively, and the distance moved is apparently a logarithmic function of time.

With repeated applications of force at regular intervals of time, such as 2–5 seconds, the tooth does not have time to return to its original position before the second and subsequent applications of force, and much of the movement which takes place on the first application of force is not immediately recovered. The gross tooth movement, therefore, is less on the second and subsequent applications of force. With each appli-

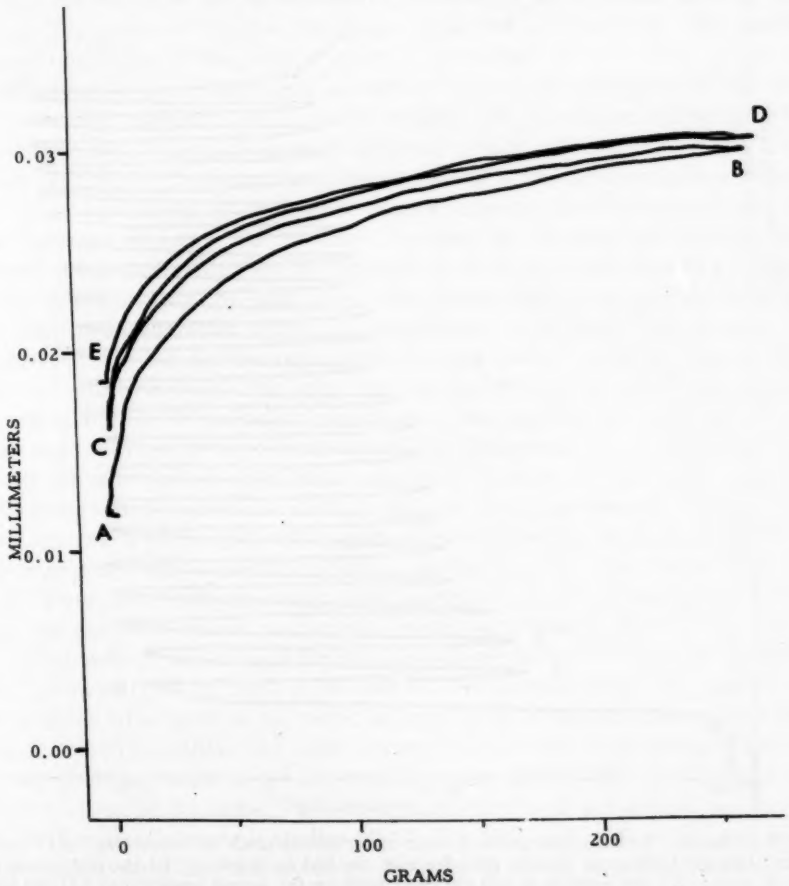

FIG. 3.—The axial force/movement curve of the same upper incisor tooth as in Fig. 2 in the evening of the same day. (*A–E* as in Fig. 2.)

cation of force the tooth becomes progressively intruded into its socket (Fig. 4). This apical displacement at peak force diminishes progressively but has been observed to reach 0.008 mm. Progressive apical displacement is dependent upon the duration of the application of force and the number of applications made during a limited period of time. The intrusive movement which takes place with each application of force and the recoil immediately following its removal are related to the force applied and not to the duration or frequency of its application. Nor are they affected by the degree of axial displacement of the tooth into its socket.

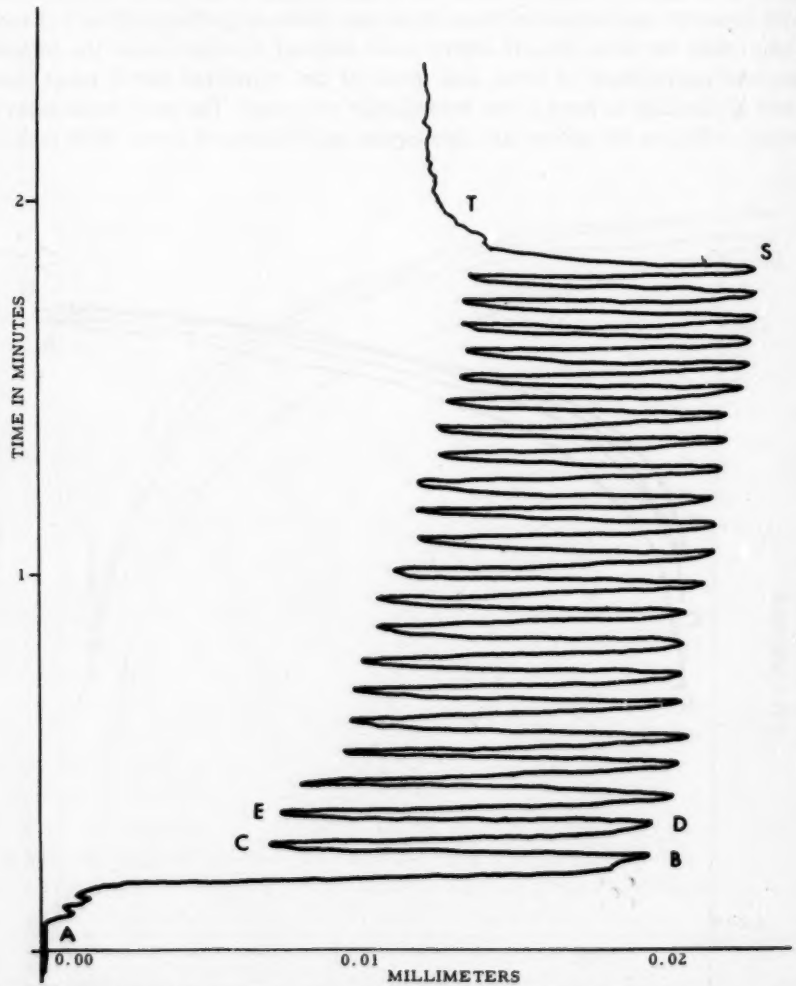

FIG. 4.—The axial time/movement curve of an upper incisor tooth in the evening. (*A*) The initial position; (*B*) the position at 500-gm. peak force on the first application; (*C*) the first return on removal of force; (*D*) the position at 500-gm. peak force on the second application; (*S*) the position at 500-gm. peak force on the twenty-second application; (*T*) the return point after the final removal of force.

2. *Continuous force of 500 gm.*—When continuous axial force is maintained, the tooth moves apically by a regular amount with each period of time (approximately 0.002 mm/min) until a limit is reached when no further displacement takes place. However, when intermittent force is repeated as much as 0.005 mm., further axial displacement may take place beyond the limit reached with continuous force.

3. *After the removal of continuous force.*—Upon the removal of continuous force, the tooth becomes extruded in two phases, as was observed when intermittent force was used; but, as the gross displacement with continuous force is greater, the duration of the second phase of return to the original position is longer. Movement of the tooth during the second phase of recovery is a logarithmic function of time, and the degree of movement is dependent upon the duration of the application of force. The first phase of immediate recoil is apparently dependent only on the force applied.

4. *Retention of a smaller force subsequent to removal of a larger force.*—Return of the tooth toward its original position during the second phase of recovery can be arrested at various points by the retention of a load upon the tooth bearing in an apical direction. The point of arrest is dependent upon the retained load. The speed of movement diminishes as the point of arrest is approached.

5. *Variations in premeasurement conditions.*—The gross movement of the tooth with known force is greatest after a period of sleep (Fig. 2) or when splints have been worn which guard the teeth from intraoral forces or after taking atropine (gr. 1/150) or ephedrine (gr. $\frac{1}{2}$). The gross movement is greatest in the morning and becomes less as the day proceeds (Fig. 3). It also decreases with heavy function or preloading with various amounts of force. The difference between the morning and evening force-movement curves is striking, but the difference appears to be due more to a change in the point of origin along the same curve after initial tooth movement has been eliminated than to an alteration of the force-movement curve itself. This is clearly the case when premeasurement traction is applied to the tooth. A traction force of 15 gm. causes the tooth to extrude 0.006 mm. from its resting position, and 30 gm. causes it to extrude 0.008 mm. When an intrusive force is then applied, the tooth first retraces its path and continues to move inward along the characteristic force-movement curve in exactly the same path as when forces were applied before traction. Other conditions have a similar effect but are not under such immediate experimental control.

Gross movement of the tooth with standard force is usually smallest in the evening but, without sleep, may continue to become smaller until morning. After sleep, however, the gross movements are larger. This condition is usual on waking in the early morning but can be produced by a period of sleep at any time. Lying down for 10 minutes without sleep at any time of the day also causes the gross movement to become larger. The mobility of the tooth in its long axis is therefore continually altering.

The position of a tooth in its socket appears to be a balance between the forces causing the tooth to extrude and those causing it to intrude. If the intraoral intrusive forces are small or absent for an appreciable length of time, the tooth takes a more extruded position in its socket than when intrusive forces are almost continuously applied. When an axial force is applied for the first time to a tooth in its usual resting position, displacement is relatively large. On removal of the force, the tooth recovers its position in 1 or 2 minutes. This initial displacement can therefore be repeated every 2 minutes; but, if a second application of force takes place within a few seconds,

recovery of position after the first application is incomplete, and the initial tooth movement is reduced or absent on the second application of force. If, before the first application of force, a small intrusive preload of 15 gm. is allowed to act on the tooth for 1 minute, the tooth becomes depressed in its socket. The initial tooth movement is also lost, and repeated applications of force meet with almost identical resistance. Three applications of a 10-gm. preload at 3-second intervals or a continuous preload of 30 gm. for 5 seconds also eliminates the initial tooth movement.

When an axial load of 0.5 gm., which is the lowest feasible contact pressure of the measurement probe against the tip of the tooth, is maintained, the tooth is observed to rise and fall synchronously with the arterial pulse wave. When the load is increased to between 2 and 4 gm., pulsation is most strong, but, with further increase in axial load, pulsation diminishes until, at 15 gm., it can no longer be detected.

DISCUSSION

The present system of measurement of axial tooth movement has proved to be a highly sensitive, accurate, reproducible, and reliable method, suitable for clinical use.

Preliminary measurements show that certain characteristics of axial tooth movement following the application of force are always present but vary in degree according to the conditions immediately preceding the measurements.

Transmission of the pulse wave by the tooth indicates that the blood vascular system affects the position of the tooth in its socket to a certain extent. Disappearance of the pulse wave and the initial tooth movement with a force of 15 gm. and the slow recovery of position and return of pulse wave and initial tooth movement when this force is removed suggest that a fluid is dissipated at this level of force. Such an ebb and flow might be explained by the collapse and recovery of the smaller blood vessels.

The most generally accepted concept is that the tooth is held by tension in fiber bundles subsequent to a small initial movement which takes up their characteristic waviness before they become taut. The finding that the tooth rises in its socket against an intrusive force of 15 gm. or more, with labial and lingual movement, would appear to confirm the idea that the tooth is slung in its socket by oblique fibers.

The characteristic displacement which takes place when forces of 30–1,000 gm. are applied to a tooth and the almost immediate recovery on their removal suggest that an elastic tissue is being compressed or put in tension or both. The relationship of movement to the logarithmic function of force is characteristic of all the soft tissues, including collagenous fiber bundles. The extent of the displacement and recoil, which varies between 0.007 and 0.016 mm., could be a deformation of the periodontal fiber bundles. However, the progressive axial displacement of the tooth in its socket to the extent of 0.007 mm. with continuous force and an even greater displacement with intermittent force make untenable the hypothesis that the tooth is directly slung in its socket by fiber bundles, particularly as the elastic recoil of the tooth is unaffected.

Axial displacement of the tooth with continuous force bearing a linear relationship with time suggests that the tooth is supported by a fluid system which, by slow displacement, allows the tooth to sink in its socket. The slow return, bearing a logarithmic relationship with time, also suggests the return of a fluid approaching an equilibrium, such as by osmotic or hydroastatic pressure. Two fluid systems seem to be involved in support of the tooth—a vascular tide below the load of 15 gm. and a tissue fluid

tide at higher loads. These tides are analogous to the blanching and pitting of subcutaneous tissue upon pressure.

Synge[12] considered that the fibers in the periodontal membrane could not be responsible for the extraordinary tightness of the teeth but that the periodontal membrane must act as a compressive elastic cushion. The characteristic displacement which takes place when force is applied to a tooth and the almost immediate recovery on its removal support this view, but the progressive axial displacement with continuous or intermittent applications of force cannot be so explained.

Gabel[13] thought that the arrangement of fibers in the periodontal membrane suggested functions other than the direct resistance to tension. He considered that, in function, certain areas of the periodontal membrane became thinned with displacement of the tooth but that, as the membrane is composed of incompressible material, lateral displacement takes place. This tangential displacement of tissue is resisted by the fibers of the periodontal membrane, which takes the form of a catenary. Such an arrangement would put tension on some of the fibers as soon as the force is applied. If part of the incompressible material of which the periodontal membrane is composed is a fluid capable of being slowly displaced by compression, then such a mechanism would explain the finding that a similar elastic recoil takes place upon removal of force in any position of apical displacement of the tooth in its socket.

Further measurements are at present being made under various experimental conditions to study the mechanisms involved in support of the tooth and to ascertain the effect on the characteristic tooth mobility of local changes in the blood vascular system, of tissue fluid changes, and of various local and systemic conditions. These will form the substance of further reports.

SUMMARY

1. A machine for the measurement of the physiological mobility of teeth, suitable for clinical use, is described. This machine utilizes rectilinear transducers and is capable of measuring axial tooth movements with an accuracy of 0.001 mm. ±7 per cent and, simultaneously, the force applied to the tooth. The time of displacement and of recovery of position of the tooth when force is removed or varied is also recorded.

2. Calibration of the machine is described.

3. Clinical measurements on one individual over a period of 12 months show that the tooth is supported by several distinct and independently variable systems. Two systems behave like displaceable fluids, and one like an elastic material.

4. Variations in mobility are described and discussed.

5. Similar force-movement curves were obtained on the teeth of several individuals.

The author wishes to thank Mr. A. L. Thomas and the staff of Southern Research Institute for their kind assistance in the design of the machine.

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Effects of Different Cutting Procedures on Dentin Apposition in Rat Incisors

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The purpose of this study was to compare the pulpal effects of cutting the dentin of rat incisors with different devices. Three different cutting instruments were used in order to discover whether the effects on the odontoblasts and the pulp were similar or different. The instruments used were a rotary bur, an air-abrasive, and an ultrasonic device.

The rat incisor was chosen as the test object for these experiments because dentin apposition is very regular and predictable and the odontoblasts are very sensitive and reactive to even minor injuries to their protoplasmic processes within the dentin.

MATERIALS AND METHODS

The lower left incisors of 69 male albino rats (50–140 days of age) were subjected to the cutting procedures. The unoperated lower right incisor was used as the control. The animals were sacrificed after 7 days, and the teeth were prepared by routine histological methods.

The animals were anesthetized by an intraperitoneal injection of a 1 per cent solution of pentothal sodium. The operations were performed under a binocular dissecting microscope under a magnification of 9 times and under good lighting conditions. The cavities were prepared on the lateral aspect of the lower left incisors. The procedure was essentially the same as that described by Mohammed and Schour.[1]

Operative procedure.—An intraoral incision was made with a No. 11 B-P blade, at the level just under the mesial aspect of the first molar, exposing the bone immediately in front of the mental foramen. Hemorrhage was controlled with cotton pellets and by topical application of epinephrine. A cavity was then prepared in the bone just anterior to and below the mental foramen with an end-cutting bur No. 959. The sudden appearance of blood indicated that the bur had reached through the bone and into the periodontal membrane surrounding the tooth. Hemorrhage was controlled by applications of epinephrine. The field was then cleaned, and the dentin thus exposed was made ready for the cavity preparation.

Bur.—Cavities were prepared in the dentin of 21 teeth, using a No. 957 end-cutting bur (2 mm. in diameter) revolving at 6,000 rpm dry. Neither air nor water was used as coolant. The cavity preparations were completed in 10–12 seconds. This represents

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a high rotary speed for cutting into rat incisor dentin, since, in previous studies, similar cavities were prepared at 300–400 rpm (dry).

Air-abrasive.—A small rubber dam was used to block off the pharyngeal area and yet leave the lower incisor exposed for the operation, to avoid the penetration of the abrasive into the lungs and choking the animal to death. The handpiece was positioned vertically 1.0 mm. above the surface of the dentin. The electrical contact was then made for exactly 1 second.

The rapidity of cutting was such that the pulps were exposed by even this brief cutting in the first series of animals. The pressure from the carbon dioxide gas tank was therefore reduced to 60 lb. to reduce the speed of cutting. One second at 40 volts and 60 lb. pressure thereafter proved satisfactory.

Ultrasonic.—The use of a rubber dam was also found to be essential during the use of the ultrasonic device, to prevent choking the animal with the abrasive slurry. Special metal tips were supplied by the manufacturer for use in these experiments, so that the size of the cavity preparations was approximately the same as that produced by the bur and the air-abrasive. The tip was held in contact with the dentin under very light pressure for 1½ seconds.

Filling material.—The cavities in the bone and in the dentin were filled with a neutral material having the following composition: zinc oxide, 2 parts; zinc sulfate, 1 part; and cornstarch, 1 part. This powder forms a paste when mixed with water and hardens rapidly. This filling has been termed "aquadont" by Orban (1934) (quoted by Mohammed and Schour).[1]

A thin layer of wax was flowed over the filling in the dentinal cavity to prevent ankylosis of the continually erupting tooth. The cavity in the bone was then filled with aquadont and the soft tissue replaced. Suturing was not possible.

Although zinc oxide and eugenol has been shown to be the least irritant temporary filling material available today and is used as a bland dressing in most experimental studies of pulpal reactions, its use was avoided, to eliminate possible criticisms in this direction. The zinc oxide–starch mixture was selected because it is non-reactive. Marginal leakage was not considered to be a factor, since the fillings were covered with wax.

The field of operation was kept clean but not sterile. In many animals the soft tissue over the site of operation became inflamed and sometimes infected.

Histologic preparations.—The animals were sacrificed after 7 days by ether, and the mandibles were dissected. The specimens were fixed in 10 per cent formalin solution or in formol–Zenker solution for 48 hours and demineralized in a 5 per cent nitric acid solution for 24 hours. A few specimens were demineralized by chelation over a period of several weeks. The specimens were imbedded in paraffin or celloidin and sectioned at 10 and 15 μ. The sections were stained with hematoxylin and eosin, silver, or with Mallory's triple connective tissue stain.

METHOD OF ANALYSIS

The effects of the various cutting procedures upon the odontoblastic cells were assessed by analyzing the quality and quantity of the postoperative dentin, as suggested by Silberkweit *et al.*,[2] rather than by direct examination of morphologic changes in these cells. Injuries to the odontoblasts which are so slight as to result in no morpho-

logic changes are readily reflected in alterations in the quality or quantity of dentin produced by these cells after surgical interferences. The quality and quantity of the postoperative dentin formed under the area of the cavity were therefore assessed by comparing them with the amount and quality of the postoperative dentin under adjacent, unoperated areas of dentin.

This method of assessing the injury to the cells by measuring the changes in the dentin produced by these cells is a very delicate but accurate measure of the altered physiology of these cells. An additional virtue is the fact that the effects can be measured objectively and do not depend on subjective estimations of morphologic alterations in the cells.

Calcio-traumatic band.—The immediate effect of cutting through the dentin and the peripheral protoplasmic extensions of the odontoblasts was the formation of a calcio-traumatic line of band which distinctly and sharply demarcated the preoperative from the postoperative dentin (Fig. 1). This effect can also be produced by experimental injections of toxic materials,[3] systemic disturbances,[4] or any operative procedure that interferes with odontoblastic activity. The odontoblasts are very sensitive to systemic or local injuries. In many specimens, the shock of the anesthesia and postoperative failure to eat for 24 hours were evidenced as a distinct calcio-traumatic line completely encircling the pulps of both the experimental and the control teeth. An accentuated calcio-traumatic band under the cavity represents *an acute arrest in odontoblastic activity as a* result of the operative procedure.[1, 2, 5]

The following notations were used for the assessment of the acute calcio-traumatic effect.

0—Normal. Calcio-traumatic effect absent or no difference between the calcio-traumatic effect under the cavity preparation and the control areas or control tooth.
1—Faint accentuation of the calcio-traumatic line under the cavity as compared to control areas; reflects a slight injury to the odontoblasts.
2—Definite accentuation of the calcio-traumatic line under the cavity; reflects a definite acute injury to the odontoblasts.

Postoperative dentin.—The amount of postoperative dentin was easily measured because the odontoblasts in these teeth deposit approximately 16 μ of dentin daily in a series of regular and rhythmic layers. The amount formed under the cavity was compared with the amount formed in the unoperated areas or in the control tooth. The measurements were taken from the calcio-traumatic line to the pulpal surface along the direction of the dentinal tubules.

The quality of the calcified tissue was also easily assessed and was graded according to the method outlined by Silberkweit *et al.*[2]

The following notations were used for the assessment of the quantity and the quality of the postoperative dentin:

Quantity

N—Normal amount of postoperative dentin, indicating an immediate and complete recovery of the odontoblasts following surgery.
1—Slight diminution in the amount of postoperative dentin formed when this was compared to non-operated areas in the same tooth and in the control tooth.
2—Definite diminution (less than half) in the amount of postoperative dentin formed under the cavity.
3—Complete or almost complete aplasia under the cavity.
4—Hypoplasia and aplasia extending beyond the area under the cavity.

Quality (Calcification of Postoperative Dentin)

N—Normal.
1—Slight degree of hypocalcification.
2—Granular or stratified postoperative dentin.
3—In some instances the disturbance was not confined to the odontoblasts alone. In some specimens hemorrhage occurred within the pulp, and in a number of specimens the pulp became filled with a peculiar osteodentin.

Cavity depth.—Since it has been established that the depth of cutting markedly influences the degree of injury to the odontoblasts, it was necessary to measure the depth of each cavity and to compare only cavities of equivalent depths. The following measurements were therefore made along the direction of the dentinal tubules, the path taken by the odontoblasts during dentin formation:

a) Depth of cutting—Measured from the surface of the tooth (dentin) to the deepest portion of the cavity.

b) Distance from the pulp—Measured from the deepest portion of the cavity to the calcio-traumatic band, the original pulpal surface at the time of operation.

Since the total thickness of dentin varied somewhat in each specimen, these two measurements were equated by dividing the amount removed by cutting (*a*, above) over the total thickness of dentin at the time of the operation (*a* and *b*, above). The cavity depths were rated as follows:

Shallow:	Less than 33 per cent of the dentin thickness removed.
Medium:	34–66 per cent dentin removed.
Deep:	67–82 per cent dentin removed.
Very deep:	More than 83 per cent of dentin removed.

RESULTS

Effects of cutting with a bur.—Under shallow cavities, the calcio-traumatic line was only faintly accentuated in all (seven) specimens of this group. The postoperative dentin was normal in amount and structure.

Under medium cavities, the calcio-traumatic line was somewhat more prominent. Only two of the five specimens in this group showed a normal amount of postoperative dentin. In three specimens marked hypoplasia was present (Fig. 1). Osteodentin formation filled half the pulp chamber in one of these three and was localized under the cavity area in the other.

Under deep cavities, the calcio-traumatic line was prominent. Postoperative dentin formation was normal in two of the five specimens in this group but markedly disturbed in the other three. In one of the latter specimens, the postoperative dentin was hypoplastic, while, in the other two, complete aplasia was noted. In one of the latter, the pulp was severely inflamed and filled with osteodentin (Fig. 2).

Under deep cavities, only one of the four specimens of this group exhibited normal postoperative dentin formation. One specimen showed hypoplastic and hypocalcified postoperative dentin. The reaction was much more severe in the other two specimens. These exhibited an aplasia localized to the area under the cavity.

Summation.—In general, the reaction under shallow cavities was the same as that previously reported by Mohammed and Schour,[1] Silberkweit *et al.*,[2] and Perreault *et al.*[5] However, the reaction under medium and deep cavities was much more severe than reported by previous investigators. In this study, hypoplasia and aplasia occurred under medium cavities in three out of five specimens (Fig. 1), whereas previous in-

vestigators reported only mild hypocalcification. Under deep cavities they reported mild to moderate hypoplasias, while this study showed severe aplasia and osteodentin formation within the pulp (Fig. 2). This was undoubtedly due to the fact that in this study the bur was revolved at 6,000 rpm (without coolant), while in previous studies the average speed ranged from 300 to 500 rpm. The higher speed was used in order to achieve a severe reaction for comparison with the effects caused by the air-abrasive and ultrasonic devices.

In general, the reactions were proportional to the cavity depth. However, the relationship between cavity depth and reaction was not so absolute as reported in previous studies by Mohammed and Schour,[1] Silberkweit *et al.*,[2] and Perreault *et al.*[5] The effects on the pulp were not consistently injurious in the medium, deep, and even the very deep cavities. Two out of five specimens with deep cavities and one out of the four with very deep cavities showed normal pulpal reactions. This is probably due to the fact that the cutting was done intermittently, with approximately four to five rests to inspect the cavity depth carefully lest the pulp be accidentally exposed. This intermittency could account for the variation in pulpal effects.

Effects of cutting with air-abrasive.—There were four specimens with shallow cavities. One specimen revealed a moderate disturbance in dentin formation (hypoplasia), and

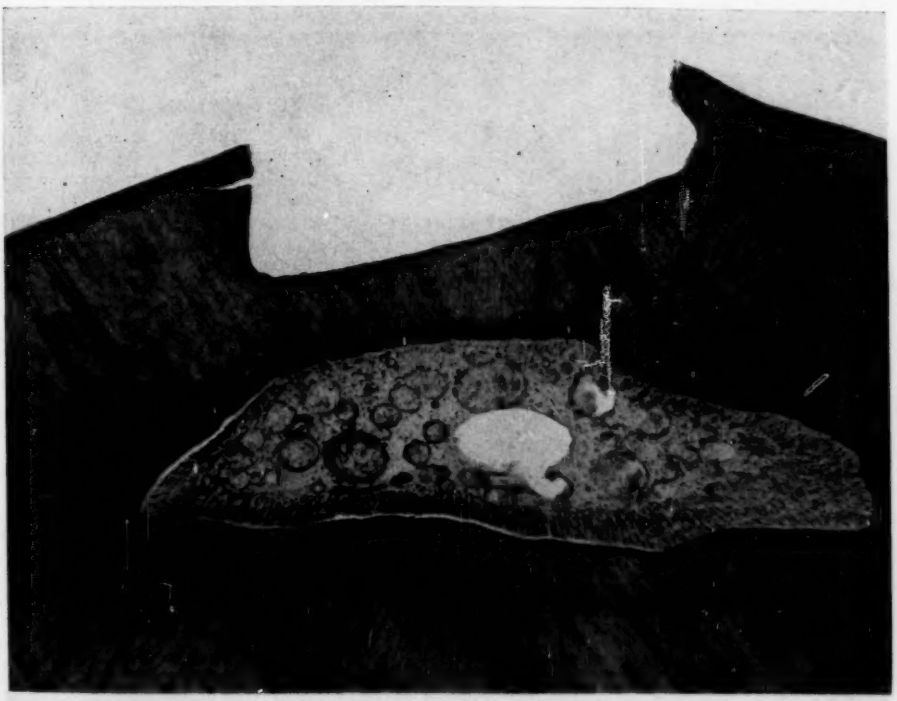

FIG. 1.—Photomicrograph of a cross-section through a rat incisor showing a severe reaction under a medium cavity prepared with a bur (6,000 rpm; dry). Note the calcio-traumatic band and hypoplasia of the postoperative dentin under the cavity. Survival, 7 days. (Hematoxylin and eosin; mag. ×68.)

in another the pulp chamber was almost entirely filled with osteodentin. The two other specimens were normal.

Cavities of medium depth were observed in nine specimens. The postoperative dentin was normal in three. Four specimens showed a marked hypoplasia (Fig. 3), while the entire pulp was degenerated in the last specimen (Fig. 4).

Only one of the five specimens with deep cavities showed a normal reaction. A slight hypoplasia was noted in one case. Hypoplasia and hypocalcification were marked in two specimens, while half the pulp was inflamed and aplastic in the last specimen.

Under very deep cavities the calcio-traumatic line was prominent. Normal postoperative dentin was seen in only one of the four specimens of this group. Hypoplasia and aplasia plus pulpal degeneration characterized the other three specimens.

Summation.—Severe damage to the pulp was apparent under more than half the cavities cut with the air-abrasive device, even under shallow cavities. In general, the disturbance tended to increase as the cavity became deeper. However, the relationship to cavity depth was not so strongly correlated as in the series cut by the bur.

Effects of cutting with the ultrasonic device.—In the six specimens with shallow cavities cut by the ultrasonic device, only two showed normal postoperative dentin formation. Four showed a marked hypoplasia and a marked hypocalcification (Fig. 5). The calcio-traumatic band was accentuated in all instances.

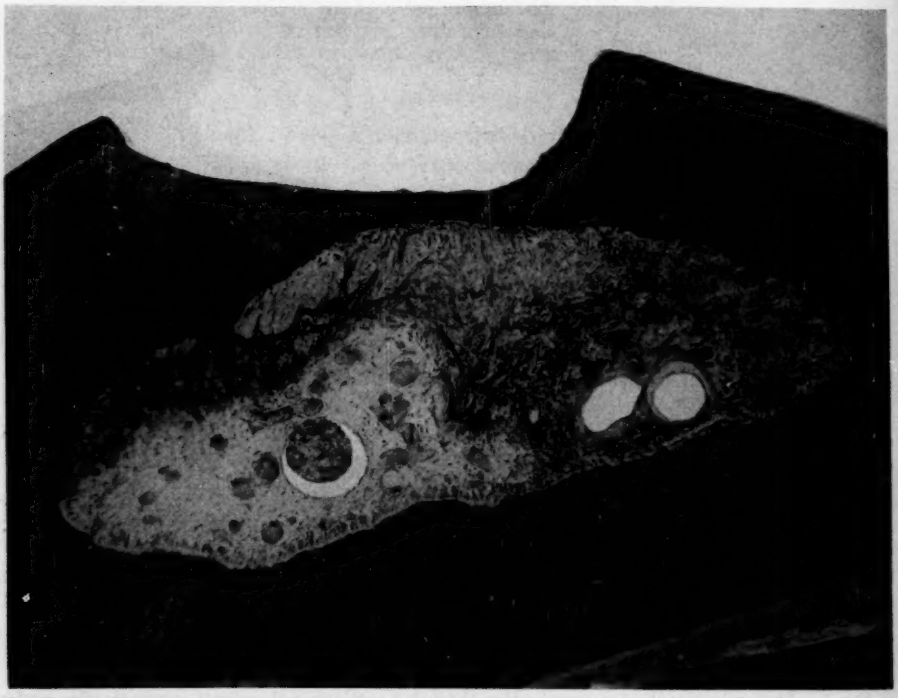

FIG. 2.—Osteodentin formation in the pulp and almost complete aplasia of postoperative dentin under a deep cavity prepared by a bur (6,000 rpm; dry). Compare with Fig. 1. (Mallory stain; mag. ×68.)

In eleven specimens with medium cavities, eight showed normal postoperative dentin formation. However, in four of these, the dentin was hypocalcified. In two specimens, hypoplasia was marked, and, in one, aplasia was present under the cavity.

There were five specimens with deep cavities. Four showed normal postoperative dentin formation, and only one showed a slight hypoplasia.

Of the four specimens with very deep cavities, all showed definite damage in the form of hypoplasia and aplasia. In one, the hypoplasia was slight; in one, it was moderate; and, in two, aplasia was complete—with pulpal inflammation.

Summation.—Damage to the odontoblasts was evident in slightly less than half the specimens, even under shallow cavities prepared with the ultrasonic device (Fig. 5). In general, the damage to the pulp increased with cavity depth. However, here again the relation was not so direct as under cavities prepared by the bur. There were more normal specimens observed under deep cavities than under medium or shallow cavities.

Pulpal scarring.—Osteodentin was noted in the pulp in seven of the sixty-nine specimens (Fig. 2). This was always associated with a generalized postoperative dentin aplasia, i.e., not localized to the area under the cavity. This represented a very severe injury to the entire pulp and not alone to the odontoblasts. Osteodentin was observed under each one of the three cutting devices and under medium as well as deep cavities. It was not confined to any one device. Osteodentin formation in the pulp was not previously described in any of the investigations reviewed.

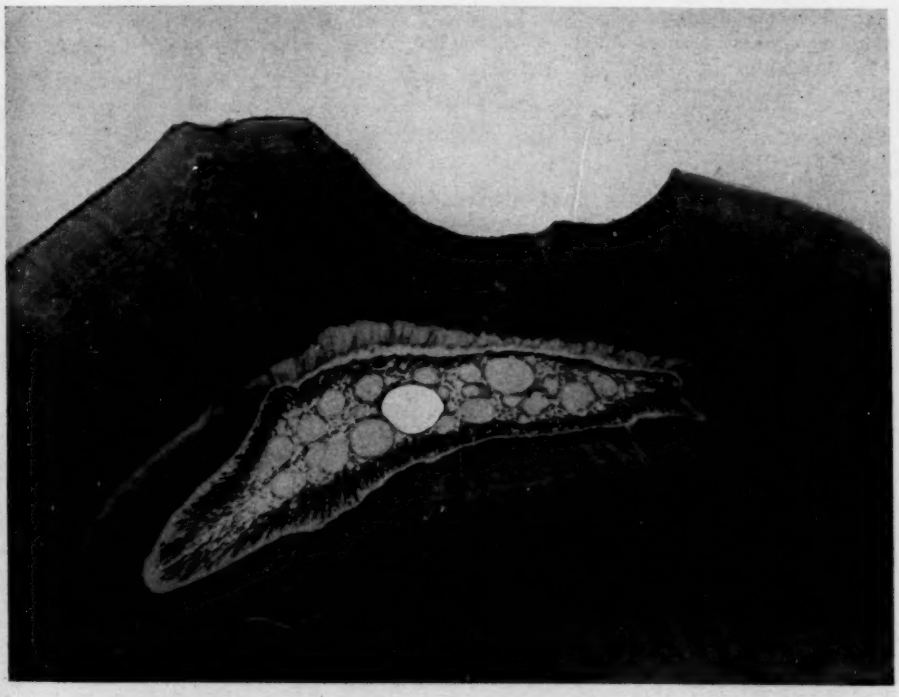

FIG. 3.—Photomicrograph of a cross-section through a rat incisor showing a severe reaction under a medium cavity prepared with air-abrasive. Note the prominent calcio-traumatic band and hypoplasia of the postoperative dentin under the cavity. Survival, 7 days. (Hematoxylin and eosin; mag. ×68.)

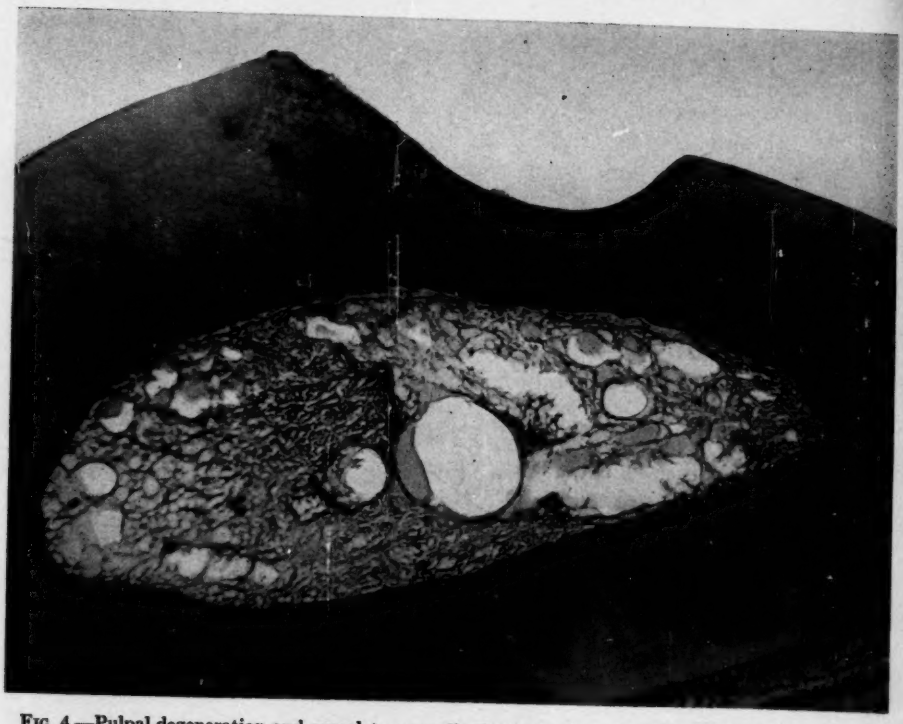

FIG. 4.—Pulpal degeneration and complete generalized aplasia of postoperative dentin after preparing a medium cavity with an air-abrasive device. Survival, 7 days. (Hematoxylin and eosin; mag. ×68.)

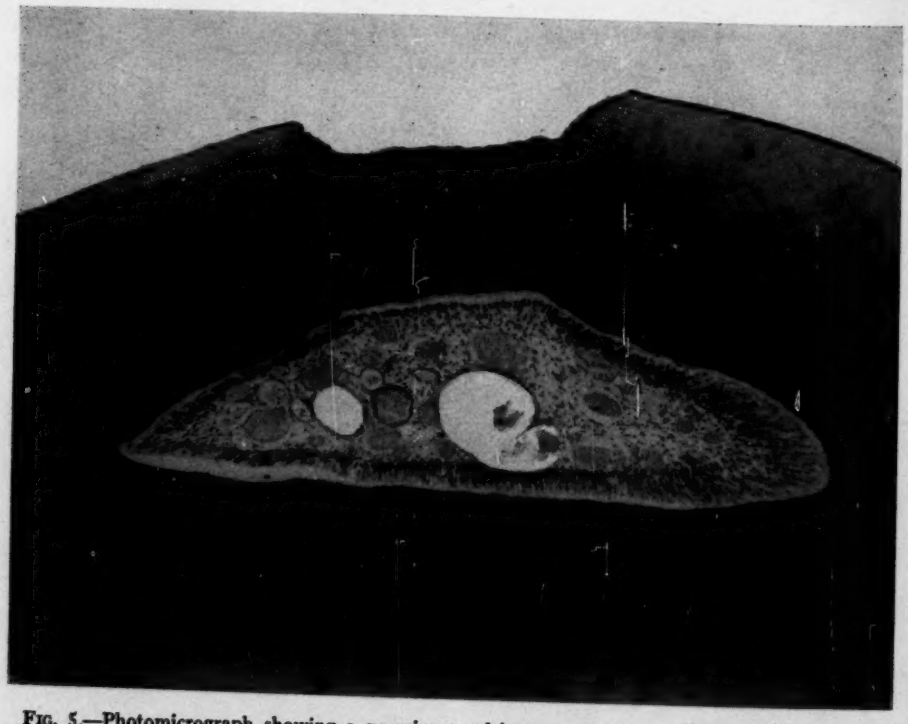

FIG. 5.—Photomicrograph showing a prominent calcio-traumatic band all around the pulp and marked hypoplasia of the postoperative dentin under a shallow cavity prepared in a rat incisor with an ultrasonic device. Survival, 7 days. (Hematoxylin and eosin; mag. ×68.)

Comparison of effects caused by the three cutting devices.—Since the depth of the cavity was important in determining the pulpal reaction, a comparison was made between the effects caused by the three cutting devices at *equivalent* cavity depths. Under shallow cavities, both the air-abrasive and the ultrasonic devices were more injurious than the bur rotated at relatively high speed in a dry field. However, when the reactions to the three devices were compared under medium cavities or under deep and very deep cavities, the numbers of specimens showing pulpal damage were not significantly different.

In general, the findings in this study confirm those obtained in other studies—that there are few differences in the pulpal reactions under cavities prepared by a bur, an air-abrasive, or an ultrasonic device.[6-12] When differences did exist, greater damage was observed under the ultrasonic device than under the bur.[13, 14]

SUMMARY AND CONCLUSIONS

This investigation was designed to study the comparative effects of cutting odontoblastic processes in rat incisors, using three different devices: a bur, an air-abrasive, and an ultrasonic instrument. The dentin and pulp of the rat incisor were chosen as the test objects because, in this tooth, dentin deposition is regular, rapid, and predictable and the odontoblasts react to injury with great sensitivity. Cavities were prepared in the incisors of sixty-nine albino rats, using three devices, and the effects were assessed by analysis of (*a*) the calcio-traumatic reaction and (*b*) the amount of postoperative dentin produced by the injured odontoblasts. Analysis revealed the following effects:

1. When cavities were cut with the bur at 6,000 rpm with a coolant, the effects were more severe as the cavity became deeper. The findings were more severe than those previously reported by Mohammed and Schour,[1] Silberkweit *et al.*,[2] and Perreault *et al.*,[5] who prepared similar cavities at much lower speeds (300–400 rpm).

2. The pulpal effects of cutting with the air-abrasive or ultrasonic device were generally not very different from, and as severe as, the effects produced by the bur. Under shallow cavities the pulpal effects were more severe under the air-abrasive and ultrasonic instruments than under the bur. No significant differences were observed under medium and deep cavities.

Photomicrographs by William Winn.

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An Analysis of Myographic Records of Swallowing in Normal and Abnormal Subjects

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Electromyography is a potential method for etiological studies of disorders of the head and neck. At present the interpretation of myographic investigations is confined mainly to qualitative impressions of muscular malfunction, since little is known of normal variation.

In the following investigation a statistical analysis has been made of electromyographic records of subjects swallowing saliva, to estimate the extent of differences between normal and abnormal types. The temporalis and masseter muscles were studied because they were easily accessible to surface electrodes. The present communication is part of a larger study involving the full range of jaw function.

Attention has been drawn by Rix[1, 2] to the relationship between the circumoral muscles and the masseter and temporalis muscles in swallowing. He studied the clinical effects of adverse oromuscular behavior on malocclusion. Tulley,[3] in an electromyographic study of children, concluded that, in normal (somatic) swallowing, the masseter muscles dominate the circumoral muscles, whereas in abnormal (visceral) swallowing the circumoral muscles tend to dominate the masseter muscles. However, no quantitative analysis of normal or abnormal swallowing was presented. The difficulty in designing such an analysis has been discussed by Campbell[4] in relation to the muscles of respiration. In this work, which was primarily anatomical, Campbell adopted a visual and arbitrary classification of no, slight, definite, and marked activity. Moyers[5] in an extensive investigation on the muscles of the head and neck, mainly of children, associated clinical findings of abnormality with the degree of muscular involvement in a particular movement.

The object of the present approach was to compare numerically records of normal and abnormal subjects. Ideally, such a comparison required the integration of the muscle action potentials arising from each muscle and movement. Electrical integration[6] was not possible on all eight channels simultaneously. Instead, the maximum potential in each quarter of a channel record was measured and the results analyzed statistically.

Our acceptance of the "integrated" muscle potential as a basis for comparison is justified by the publications of Lippold[7] and Bigland and Lippold,[8] who showed that the integrated electrical activity of the myogram was linearly related to the force and velocity of the contraction. When this relationship is considered with the unique bilateral disposition of the jaw musculature, the possibility of successfully investigating abnormality by this method increases.

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EXPERIMENTAL METHODS

In this study a normal subject was defined as one without symptoms or gross signs of oral malfunction; all normals were students or staff of the Royal Victoria Hospital. Out-patients seen routinely in the Dental Department provided the abnormal series.

Twenty-four subjects, 6 from each of the four types, normal males, normal females, abnormal males, and abnormal females, who had provided the best-quality records were selected from more than 60 individuals for whom traces were available. Table 1 lists the ages in years of the 24 chosen subjects by type, oral classification, and, for the "abnormals," by diagnosis.

TABLE 1

CHARACTERISTICS OF THE SAMPLE

Oral Classification	Age in Years of Subjects				Diagnosis of Abnormals
	Normals		Abnormals		
	Male	Female	Male	Female	
Skeletal 1:					
Occlusal 1	21, 21	19, 23, 28, 32	18, 22, 32	19, 20, 22, 22	Temporo-mandibular joint arthrosis Unilateral masseteric hypertrophy
Occlusal 2, division 1	21				
Occlusal 3	23				
Skeletal 2:					
Occlusal 2, division 1	38	19	26	11	Bilateral mandibular hypoplasia
Skeletal 3:					
Occlusal 1	21	22			
Occlusal 3			18, 35		Bilateral manidbular hyperplasia
				28	Temporo-mandibular joint arthrosis
Median age	21	23	24	21	

Recordings were made on an eight-channel electroencephalograph at an amplification of 50 μv = 5 mm. The frequency limit was 75 cps with a time constant of 0.03 sec. The recording speed was 1.50 cm/sec.

After careful skin preparation, bipolar surface electrodes, 1.50 cm. apart, were positioned over the anterior and posterior fibers of the temporalis muscle and the superficial and deep fibers of the masseter (Fig. 1). The interelectrode resistance was always less than 50,000 ohms and generally below 20,000 ohms. An earth electrode was applied on the posterior cervical region. Standard saline and electrode jelly were used as electrolytes.

Simultaneous bilateral recordings were made with the arrangement of electrodes and channels as shown in the accompanying table. The recordings were made with the patient seated and looking directly toward the front with the head unsupported.

Muscles	Channels		Electrodes
	Right Side	Left Side	
Anterior temporalis.....	1	5	Silver disks*
Posterior temporalis.....	2	6	Saline pads†
Deep masseter.....	3	7	Rubber suction‡
Superficial masseter.....	4	8	Rubber suction‡

* Ediswon. † Ediswon. ‡ E.M.I.

To reduce the eight-channel electromyograph of a swallow to simple numerical terms, the time base of each record was divided into four, and the maximum potential in each quarter was measured to the nearest millimeter. The mean of these four values in each channel was approximately equal to the activity of the corresponding muscle. This assumed that the traces in each channel had symmetrical envelopes and were of

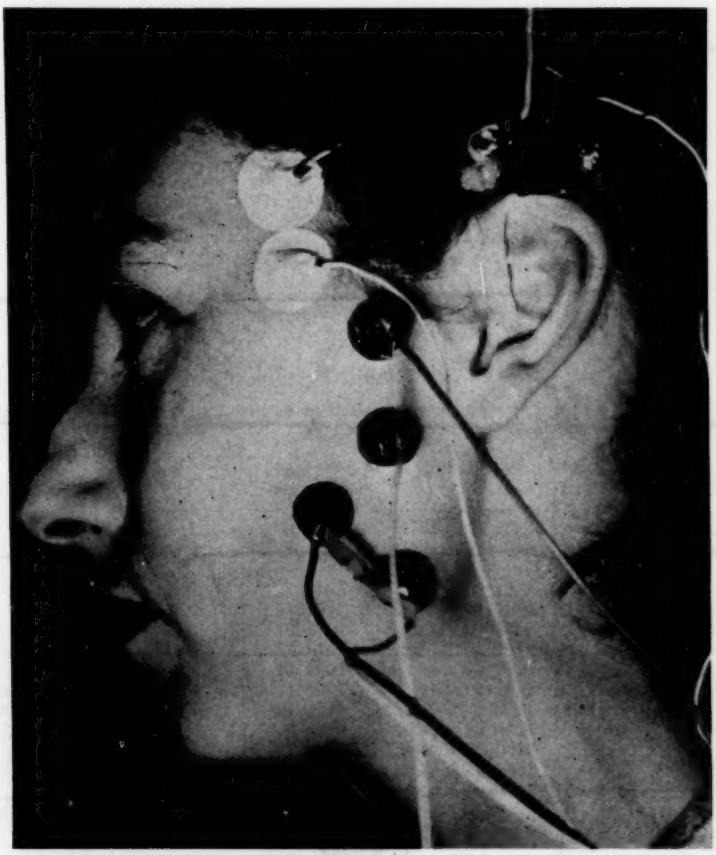

Fig. 1.—Photograph to show position of electrodes

the same duration. Two swallow records for each of the 24 selected subjects were measured.

To test whether the duration of swallowing varied between types, an analysis of variance was done on the 48 observed durations. Table 2, which summarizes this analysis, indicates at the 5 per cent significance level that there were no significant differences in durations between the four types of subject or between individually repeated swallows (replications). Significant differences occurred between the mean durations of subjects, but, since there was no evidence that these were due to functional abnormality or sex, the representation of muscular activity by maximum action potentials was warranted.

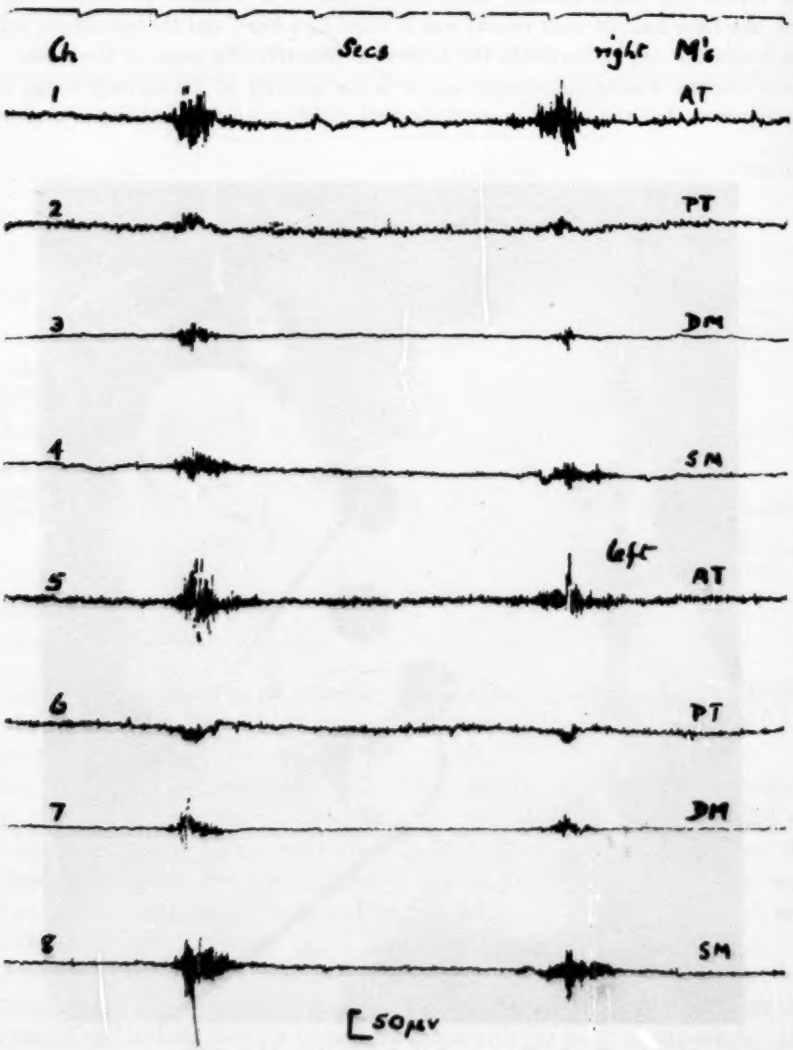

FIG. 2.—Two examples of the voluntary swallowing of saliva from the patient shown in Fig. 1

The mean duration of swallowing in these subjects was 2.02 seconds, with a standard deviation of 0.51 second. The observed range was from 0.93 to 3.20 seconds. The standard deviation of differences between durations of repeated swallows was 0.39 second.

MUSCULAR ANALYSIS

The muscular activity in each subject was represented by 64 measurements. These were the heights of the maximum peaks in the quarters of eight channels from two swallow records. As the heights of the peaks were non-normally distributed, they were transformed to logarithms to the base 10. For convenience, values under 1 mm. were given a logarithm of zero. This was equivalent to raising the base line by 1 mm., below which level valve noise and difficulties in measurement led to unreliable estimates.

An analysis of variance[9] was made with logarithmic data. From Table 3 it can be

TABLE 2

ANALYSIS OF VARIANCE OF OBSERVED SWALLOW DURATIONS IN MILLIMETERS WHERE 15 MM. = 1 SEC.

Source of Variation	Sum of Squares	D.F.	Mean Squares	Variance Ratio	n_1, n_2	P*
Between types	278.56	3	92.85	1.15	3, 20	—
Within types	1615.42	20	80.77			
Subjects	1893.98	23	82.35	2.38	23, 20	*
Replications	0.53	1	0.53	<1	1, 20	—
Types × replications	69.58	3	23.19	<1	3, 20	—
Residual	693.39	20	34.67			
Total	2657.48	47				

* Significant at the 5 per cent level. Probability $P < 0.05$. — Not significant at the 5 per cent level. $P \geq 0.05$.

TABLE 3

ANALYSIS OF VARIANCE OF LOG_{10} OF MAXIMUM SIGNAL IN E.M.G. SWALLOW RECORDS

Source of Variation*	Sum of Squares	D.F.	Mean Square	Variance Ratio	n_1, n_2	P†
Between types	0.5135	3	0.1712	<1	3, 23	—
Within types, error (a)	25.4732	20	1.2737			
Subjects	25.9867	23	1.1299	17.63	23, 768	**
Muscles	21.3392	7	3.0485	7.55	7, 140	**
Muscles × types	10.3410	21	0.4924	1.22	21, 140	—
Error (b)	56.5494	140	0.4039			
Quarters	14.1900	3	4.7300	70.60	3, 480	**
Quarters × types	1.5294	9	0.1699	2.54	9, 480	**
Quarters × muscles	1.4731	21	0.0701	1.05	21, 480	—
Quarters × muscles × types	2.6980	63	0.0428	<1	63, 480	—
Error (c)	32.1489	480	0.0670			
Replications	49.2036	768	0.0641			
Total	215.4593	1535				

* The components of error (b) are just significantly different ($0.05 > P \gg 0.02$) by Bartlett's test (see Ref. 11). It is assumed that its use in testing the muscle and muscle × type effects is warranted.

† ** = Significant at the 1 per cent level ($P < 0.01$). — Not significant at the 5 per cent level ($P > 0.05$).

seen that highly significant differences ($P < 0.01$) were found in mean values of activity (a) between the four quarters and the sixteen-quarter types combinations, (b) between the eight muscles, and (c) between the 24 subjects. None of the remaining sources of variation reached the 5 per cent level of significance.

a) Quarters.—The quarter means were arranged in increasing order for each type of subject (Table 4). The "new multiple range test"[10] was used. By this method, mean heights not significantly different at the 5 per cent level are underscored. The second quarter had the greatest mean activity over all muscles and for each type of subject but was not significantly greater than the third quarter, which had the second highest mean in each type. The second and third quarter means were significantly higher than the first and fourth quarters. The significant quarter x type interaction seen in Table 3

TABLE 4

RANKED MEANS OF LOG_{10} OF MAXIMUM SIGNAL FOR QUARTERS BY TYPE

Types	Quarters (1, 2, 3, 4) Ranked from Lowest to Highest*			
Normal males	4	1	3	2
	0.3144	0.4139	0.5932	0.6161
Normal females	1	4	3	2
	0.3436	0.3946	0.5035	0.5307
Abnormal males	4	1	3	2
	0.3440	0.4115	0.5136	0.5824
Abnormal females	4	1	3	2
	0.3260	0.4714	0.5647	0.5934

* Means underscored are NOT significantly different at the 5 per cent level. Standard error = 0 0264.

was presumably due to the differences between types in respect to the lowest two quarter means. From Table 4 it can be seen that, whereas the first and fourth quarters were not significantly different for normal females and abnormal males, the first quarter mean was significantly greater than the fourth quarter mean for normal males and abnormal females. This apparent sex reversal—abnormals of one sex giving a pattern of quarter means more like those of normals of the opposite sex than any other type—was not readily applicable. Future analysis of records of this and other movements may demonstrate the effect to be spurious. Our requirement of symmetrical muscular activity in the swallow to estimate total muscular activity would appear to hold for normal females and abnormal males (quarters 1 and 2 not significantly different from quarters 4 and 3, respectively), but only approximately for normal males and abnormal females (quarter 4 significantly less than quarter 1).

b) Muscles.—For this reason and because there was significant heterogeneity in the components of error b (Table 3), differences between the mean activities of muscles were examined separately for each type of subject. Table 5 shows the ranked means of muscles according to type of subject. As in Table 4, Duncan's test was used to detect differences significant at the 5 per cent level using the appropriate standard error for each type. The combined variance for normals was significantly less than that for ab-

normals, suggesting greater variability in the total muscle activity of abnormals. There were 10, 11, 6, and 0 significant differences between muscle means in normal males, normal females, abnormal males, and abnormal females, respectively. This trend was partly due to the size of the standard errors of the four types of subject.

The most important conclusion to be drawn from this analysis is that there was symmetrical bilateral activity of the temporalis and masseter muscles in swallowing. This is clear from Table 5, where muscle pairs (represented by channel numbers 1,5: 2,6: 3,7, and 4,8) are seen to be underscored by an unbroken line. In no pair, therefore, was one side significantly more active than the other in any type of subject.

TABLE 5

RANKED MEANS OF LOG_{10} OF MAXIMUM SIGNAL FOR MUSCLES BY TYPE

Type	Standard Error	Muscles (Channels 1–8) Ranked from Lowest to Highest							
Normal males . . .	0.0810	7 0.2088	3 0.2760	1 0.4037	6 0.5161	8 0.5494	4 0.5955	5 0.6354	2 0.6903
Normal females . .	0.0702	7 0.1791	3 0.2110	1 0.3890	5 0.4815	4 0.4987	8 0.5482	6 0.5694	2 0.6681
Abnormal males . . .	0.1009	7 0.2066	3 0.2443	5 0.4202	6 0.4598	1 0.5210	4 0.6057	2 0.6201	8 0.6254
Abnormal females . .	0.1095	5 0.3684	1 0.4290	3 0.4293	4 0.4583	2 0.4956	6 0.5345	7 0.5538	8 0.6424

Because of this symmetry, it was possible to combine the means of muscle pairs. These are presented in Table 6 in terms of the original measurement, as are the relevant standard errors. Again a continuous line was drawn underneath means judged to be not significantly different at the 5 per cent level by Duncan's test.

The posterior temporalis muscles (channels 2 and 6) and the superficial masseter muscles (4 and 8) had the two highest means. Since neither was significantly lower than the other in any type, the change in ranking from normals to abnormals (Table 6) is of no clinical importance. The anterior temporalis muscles (1 and 5) had the third highest means except in abnormal females. Only in one type, that of normal females, was the anterior temporalis significantly lower than the highest mean. The lowest mean, significantly so in all but abnormal females, was generally due to the deep masseter muscles (3 and 7).

Since there was no clear evidence of differences in muscular involvement by type of subject, it is concluded generally that the posterior temporalis and superficial masseter muscles predominate in the swallow, with the anterior temporalis having an activity just less than these two, but demonstrably greater than that of the deep masseter.

c) Subjects.—The analysis has shown (Table 3) that, although there were highly significant ($P < 0.01$) differences between mean activities in the 24 subjects, there were no significant differences between the means of the four types of subject. As the age and oral classification of those participating in the study had been recorded (Table 1), it was possible to examine whether these factors had any influence on a subject's mean activity. Because of the very small numbers in most oral subdivisions, skeletal and occlusal classifications were examined separately. No significant difference was found between the means of the three skeletal types or between the three occlusal

TABLE 6

RANKED MEANS OF MAXIMUM SIGNAL IN MILLIMETERS FOR MUSCLE PAIRS BY TYPE OF SUBJECT

Type	Muscle Pairs Ranked from Lowest to Highest				Standard Error (Mm.)
Normal males	3 and 7 1.75	1 and 5 3.31	4 and 8 3.74	2 and 6 4.01	1.06
Normal females	3 and 7 1.57	1 and 5 2.72	4 and 8 3.34	2 and 6 4.16	1.05
Abnormal males	3 and 7 1.68	1 and 5 2.96	2 and 6 3.47	4 and 8 4.13	1.17
Abnormal females	1 and 5 2.50	3 and 7 3.10	2 and 6 3.27	4 and 8 3.55	1.18

means. The correlation coefficient between age and subject activity was negative but was not significantly different from zero. These findings indicate that there is no strong association between a person's muscular activity, as measured here for the swallow, and his oral classification or age. The numbers, however, were so small and the age range so narrow that the possibility of weaker associations still exists.

SUMMARY AND CONCLUSIONS

The action of the masseter and temporalis muscles (in 12 normal and 12 abnormal subjects, 6 of each sex) during the swallowing of saliva has been recorded electromyographically. Analysis of two swallow records for each of the 24 subjects led to the following conclusions:

1. Significant differences in the durations of swallows occurred between subjects. These differences were not due to sex or to abnormal function. The observed range of durations was from 1 to 3 seconds, approximately.

2. Significant differences in total recorded muscular activity occurred between subjects. These similarly could not be attributed to type of subject (normal or abnormal, males or females) or to oral classification or age.

3. The swallowing of saliva involved the symmetrical activity of bilateral masseter and temporalis muscles in each type of subject.

4. Muscles giving the highest average activity in swallowing saliva were jointly the posterior fibers of the temporalis and the superficial fibers of the masseter.

5. With the exception of abnormal females, the activity of the deep masseter was significantly less than that of the other three bilateral muscles in the swallow.

6. Average muscle activity was significantly lower in the last quarter of a swallow than in the first for normal males and abnormal females. This suggested a tendency for abnormals to have a swallow pattern resembling that of normals of the opposite sex.

7. In general, no difference was found in the level or type of activity between normals and abnormals that was sufficiently large and consistent to permit discrimination between these types solely on the grounds of their swallow records.

The authors are indebted to the recordists, Miss A. Coey and Miss W. Macmillan; to the Photographic Department of the Institute of Clinical Science; and to Mrs. D. Hutton for preparing the typescript.

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The Effect of Phosphates on the Acid Fermentation of Sugar Saliva Mixtures

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It has been reported by several investigators that various phosphates and ashed foods added to cariogenic diets decreased the cariogenicity of the diets. The effects of the orthophosphates have been explained on the basis of mass action. However, the more effective meta- and pyrophosphates cannot be explained on this basis. In view of this it was thought of interest to determine whether phosphates could modify the rate of acid formation in sugar-saliva mixtures.

Twenty-six phosphates including ortho-, meta-, pyro-, and cyclic varieties were tested.* Only the meta-, pyro-, and cyclic varieties were effective in inhibiting acid formation (see table). In each case,

THE PH OF THE FERMENTING MIXTURE

	Hours							
	0	1	2	3	4	5	6	7
Test material 100 mg/100 ml:								
Control (saliva+sucrose)	7.7	7.0	6.3	5.2	5.0	4.8	4.8	4.7
Sodium hexametaphosphate	7.6	7.8	7.9	7.9	7.8	7.6	7.4	7.4
Trisodium pyrophosphate	7.4	7.6	7.6	7.5	7.3	7.0	6.8	6.7
Sodium tripolyphosphate	7.8	8.1	8.3	8.4	8.4	8.4	8.3	8.3
Tetrasodium pyrophosphate	7.9	8.2	8.1	7.9	7.7	7.5	7.3	7.1
Tetrapotassium pyrophosphate	8.1	7.5	7.3	6.9	6.7	6.5	6.4	6.4
Potassium metaphosphate	7.8	7.2	7.0	6.7	6.6	6.4	6.2	6.0
Potassium tripolyphosphate	7.9	7.7	7.8	7.7	7.6	7.6	7.5	7.5
Sodium acid pyrophosphate	7.2	7.3	7.3	7.2	6.9	6.7	6.5	6.5
Victamide	7.3	7.2	7.2	7.3	7.2	7.1	7.0	6.9
Test material 50 mg/100 ml:								
Control (saliva+sucrose)	7.8	7.0	6.5	5.3	5.0	4.8	4.6	4.6
Sodium hexametaphosphate	7.7	7.6	7.4	7.2	7.1	6.9	6.7	6.7
Trisodium pyrophosphate, anhyd.	7.6	7.5	7.3	7.1	6.9	6.6	6.4	6.5
Sodium tripolyphosphate, anhyd.	7.9	7.8	7.8	7.6	7.6	7.4	7.3	7.3
Tetrasodium pyrophosphate, anhyd.	8.1	7.8	7.6	7.3	7.1	6.8	6.6	6.7
Tetrapotassium pyrophosphate, anhyd.	8.1	7.6	7.3	7.1	6.9	6.6	6.4	6.5
Potassium tripolyphosphate, anhyd.	7.9	7.6	7.5	7.2	7.1	6.9	6.8	6.8
Sodium acid pyrophosphate	7.3	7.4	7.4	7.1	6.9	6.7	6.4	6.4
Victamide	7.8	7.7	7.7	7.6	7.5	7.3	7.2	7.3

a 1 per cent solution of sucrose in caries-active saliva with and without the test material was allowed to ferment for 7 hours. At hourly intervals the pH was taken. If the pH did not drop below 6.0, the test material was considered to have exerted some inhibitory activity.

The meta- and pyrophosphates were effective in a concentration of 100 mg/100 ml. Eight of these showed inhibitory activity in a concentration of 50 mg/100 ml. None showed appreciable activity at concentrations of 25 mg/100 ml. The table shows the degree of inhibition of those with appreciable inhibitory activity at 100 mg/100 ml and 50 mg/100 ml.

On the basis of these results it is possible that the caries-reducing activity of the meta- and pyrophosphates may be associated with their acid-inhibiting activity.

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* All the salts were furnished by the Victor Chemical Company.

An Automatic Water Supply for Rodents

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Provision of water for large numbers of rodents from bottles is probably the most time-consuming task in an animal laboratory. Even with diligence, the occasional animal is deprived of water because of oversight or mechanical failure.

The design and installation of an automatic water system has increased our over-all efficiency by major reductions in labor and prevention of accidental water deprivation. A low-pressure main was brought into each room to provide a carefully regulated and adequate flow of water at 4–5 psi. Valved branches were provided to connect the racks of cages. The plumbing system on each rack was planned so that a water fount* projects a short distance through a hole 7/8 inch in diameter at a standard height in the panel that forms the back of the cage. The plumbing on each rack is attached from its highest pipe to a higher branch on the low-pressure main by clear, flexible plastic tubing, to permit mobility and for observation of the collection of air bubbles. Manually operated valves are located at strategic points to release air that accumulates because of the reduced solubility of air at reduced pressure or the recent connection of a clean rack. Little contamination can occur because, unlike the use of water from bottles, water usage from the founts is replaced from the main without the retrograde passage of air. A minimum leakage from the founts occurs if the pressure is carefully adjusted, if clean filtered water is used to prevent particles lodging at the seal in the fount, and if the design insures that neither the food cup nor any part of the cage can rest against the fount. Although we use city water passed through a filter and reducing valve, distilled water could be supplied from a storage tank at an appropriate height.

To insure that this automatic system would not interfere with our caries assays, an experiment was conducted with caries-susceptible rats fed ration 2700 (J. H. Shaw and D. Griffiths, *J. D. Res.*, **39**:377, 1960). One group of 32 rats was provided with water by conventional bottles, while another group of 35 littermates was provided water from the automatic system. After 70 days, the average number of carious molars and average extent of carious lesions for group 1 were 5.3 ± 0.5 and $26+ \pm 4+$, respectively, and 5.1 ± 0.4 and $23+ \pm 3+$, respectively, for group 2. The growth rate, general health, and well-being of the rats were excellent with no distinction between groups.

The use of this automatic water system has been compatible with our procedures for caries assays and with the maintenance of health and vigor in our breeding stock and experimental groups. With our population of approximately 1,000 rats, the system results in about 4 hours less labor per day.

This study was supported in part by Research Grant D-204 from the N.I.D.R., U.S. Public Health Service.

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* H. W. Hart Manufacturing Company, P.O. Box 3456, Glendale 1, California.

The Effect of Cocoa Ash on Caries in the Rat

Comparison of Ashed Cocoa with a Mineral Mixture

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A previous study in desalivated rats suggested a caries-reducing effect of cocoa beans and cocoa fractions when added to cariogenic diets (Rozeik, Cremer, and Hannover, *Deut. zahnärztl. Z.*, **11**:1104, 1956). The various observations of a caries-reducing effect of ashed foodstuffs (Sognnaes and Shaw, *J. Nutrition*, **53**:195, 1954; Nizel and Harris, *J. D. Res.*, **34**:513, 1955; Büttner, Cremer, and Hartung, *Deut. zahnärztl. Z.*, **12**:541, 1957; Büttner and Muhler, *J. D. Res.*, **38**:1052, 1959) raised the question as to whether certain trace elements, on the one hand, or the mineral mixture present in the ash, on the other, was responsible for the caries-reducing action. The present study deals with the effect of (*a*) cocoa ash, (*b*) neutralized cocoa ash, and (*c*) a mineral mixture, comparable to the minerals present in cocoa ash, upon the caries incidence in the rat.

Groups of 20 weanling rats of the Wistar strain received cariogenic diet "Stephan 580" alone, or the same diet and cocoa ash (pH 10), or neutralized cocoa ash (pH 7) at a level of 2 per cent each for experimental periods of 20, 40, 60, 120, or 180 days, respectively. Two other groups of 20 rats each received "synthetic" ash (pH 6.7), comparable to the mineral composition of the cocoa ash, at a level of 2 per cent for 20 and 40 days, respectively. The composition of this mixture was as follows: K_2O, 41.9 per cent; Na_2O, 2.8 per cent; CaO, 5.2 per cent; P_2O_5, 41.0 per cent; CO_2, 8.7 per cent; and Cl, 0.4 per cent. The following reagent-grade salts had been used: KH_2PO_4, K_2HPO_4, K_2CO_3, Na_2CO_3, $CaCO_3$, and NaCl.

Caries evaluation was carried out in undecalcified ground sections of the molar teeth (Marthaler, Mühlemann, and König, *Schweiz. Z. Zahnheilk.*, **67**:755, 1957) after experimental periods of 20, 40, and 60 days. The caries incidence of rats remaining 120 and 180 days on the respective diets was determined acording to the method of Muhler, Nebergall, and Day (*J. D. Res.*, **33**:33, 1954).

The addition of 2 per cent cocoa ash to the diet resulted in a significant caries reduction of 98 per cent ($t = 4.7$) after 20 experimental days. After 40 days, however, the caries reduction was only numerical and not significant.

Adding neutralized cocoa ash to the diet caused a lasting caries-reducing effect through the entire experimental periods up to 180 days, at which time the caries reduction was found to be 67 per cent ($t = 2.6$).

The effect of adding a mineral mixture comparable to the mineral composition of cocoa beans upon the caries incidence as compared to the controls, the cocoa-ash group, or the neutralized cocoa-ash group, respectively, was observed over experimental periods of 20 and 40 days. At the termination of the experiment after 40 days, the mineral mixture had caused the same caries reduction as the neutralized cocoa ash. The mineral mixture was composed of reagent-grade chemicals. Therefore, it seems unlikely that the caries-reducing effect of cocoa ash is due to the presence of certain trace elements. The caries-reducing action of cocoa as well as of other ashed foodstuffs seems to be mediated rather by the presence of a mixture of phosphates, chlorides, and carbonates, as recently suggested by McClure and Muller (*J. D. Res.*, **38**:776, 1959).

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Abstracts of Papers Presented at Meetings of the Boston Section

1. *Osteogenic Induction by Extracts of Nonvital Bone and Other Tissues in the Rat.*—MELVIN L. MOSS (*College of Physicians and Surgeons, Columbia University; October 23, 1959*). Incubation of non-vital bone paste at 37° C. in Ringer-Tyrode solution extracted a factor which constantly induced osteogenesis when implanted intracerebrally, in saturated Gelfoam, in 32 young Long-Evans rats. Host response in 15 days consisted of primary formation of isolated ossicles and secondary endocranial trabeculae within the implant area exclusively. Chemical analysis demonstrated chondroitin sulfate in the osteogenetically active extracts. This recovered material when redissolved and similarly implanted, constantly induced osteogenesis in 12 rats. Identical but inconstant responses followed implantation of extracts of cat bladder, dog skeletal and cardiac muscle, and acid-pepsin–digested powdered bovine traceal cartilage. Untreated bone paste produced only sutural synostosis. After extraction, the paste residue evoked an intense localized osteogenic response in the host. The former responses were observed in only 1 of 25 controls. Host reaction to operative trauma was unequivocally different. Current definitions and several theoretical mechanisms of induction were reviewed. Tentatively, the matrix interaction theory of Grobstein was favored and the inductive factor identified as a mucoprotein or a mucoprotein-polysaccharide complex.

2. *Problems of Regeneration and Repair of Mineralized Tissues.*—RICHARD J. GOSS (*Brown University; November 18, 1959*). The growth mechanism of skeletal tissues is conveniently available for investigation in regenerating structures which recapitulate their embryonic development following injury. This phenomenon is readily observed in many of the lower vertebrates following amputation of appendages. Replacement is accomplished by the aggregation of undifferentiated cells at the end of the stump and subsequent differentiation into specialized tissues. This process has been studied extensively in many different kinds of appendages and intensively by a variety of experimental technics.

From the comparative viewpoint, many different types of skeletal regeneration have been investigated. In fishes the deposition of new scales to replace those which have been lost and the differentiation of bony fin rays in regenerating fins have been studied. The production of cartilage and bone in regenerating limbs, tails, and jaws of amphibians has also been investigated, as has the *de novo* formation of teeth in regenerating salamander jaws. Even in mammals, regeneration is well illustrated by the repair of fractured bones and the annual replacement of antlers in deer.

In addition to direct observations, the process of skeletal regeneration has been analyzed experimentally. Techniques involving the extirpation or transplantation of tissues have yielded interesting results, and irradiation has proved a useful method of attacking certain problems. By such methods, valuable information has been obtained concerning the mechanisms by which skeletal tissues normally develop, frequently repair injuries, and occasionally replace themselves.

3. *An Excursion through the Ultrastructure of Calcified Tissues.*—ROBERT M. FRANK (*Dental Institute, Faculty of Medicine, Strasbourg, France; December 16, 1959*). Bones and teeth have been studied by means of the electron microscope since 1943. More recently, technics have greatly improved, and it is now possible to study these structures, including mature enamel, by the direct examination of ultra-thin, non-decalcified sections. Thus, on the same preparation, it is possible to make electron-microscopic and crystallographic observations.

In mature human enamel, the structure of the prism sheath, the prism core, and the interprismatic substance can be accurately examined. In addition, apatite crystal orientation, differences in mineralization of adult enamel, morphology of enamel crystals, and especially organic and inorganic relationships can be explored. It appears that the enamel crystals are not contained within the spaces of the fibrillar network, as is generally believed, but that the fibrils run through the substance of the crystals.

In dentin the relationship of the odontoblastic processes, the dentinal tubules, and the intertubular substance has been investigated.

During dental caries, the process of destruction and bacterial invasion of the dental hard tissues has been followed. Pathological crystallizations were observed in the translucent carious zone of dentin.

In bone investigations, special attention was given to the fibrillar architecture, and two types of lamellar bone were seen.

Electron diffraction patterns from human calcified tissues show that the crystals of both bone and teeth belong to the apatite group. However, there are great variations in the size of the enamel, dentin, and bone crystals. Because of the small size of bone crystals, it has not been possible until now to obtain a diffraction pattern from a single crystal. As enamel crystals are the largest of the body, single crystals have been isolated under the electron microscope, and single crystal diffractions have been achieved.

4. *Production of Oral and Gastrointestinal Lesions after Stimulation of the Central Nervous System.*—GIULIANO QUINTARELLI (*Tufts University School of Dental Medicine; January 20, 1960*). The purpose of the present investigation was to determine the effect of chronic visceral brain-stem stimulation on the oral cavity. Experiments were performed on 17 dogs in which the stimulus was applied by two different procedures. Seven animals received traumatic irritation by means of a blunt probe. Ten animals were stimulated with glass beads located in the sella turcica, which produced a mild continuous stimulation on the tuber cinereum, infundibulum, and mammillary bodies. Six animals served as controls and were operated in the same manner as the experimental animals which received no cerebral stimulation. All animals in the first group died by the eleventh postoperative day, showing severe hemorrhage of the gastric and intestinal mucosa and superficial erosion of the intestinal tract. No oral lesions were encountered. Six animals of the second group showed changes in the oral cavity. These changes were bleeding, ulcerous stomatitis, and periodontal disturbances. This latter group also exhibited gastric bleeding, focal lesions, and ulcers in duodenum and jejunum. Control animals kept in the same environment showed neither changes in the oral cavity nor in the intestinal tract.

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